JOURNAL OF DAIRY SCIENCE

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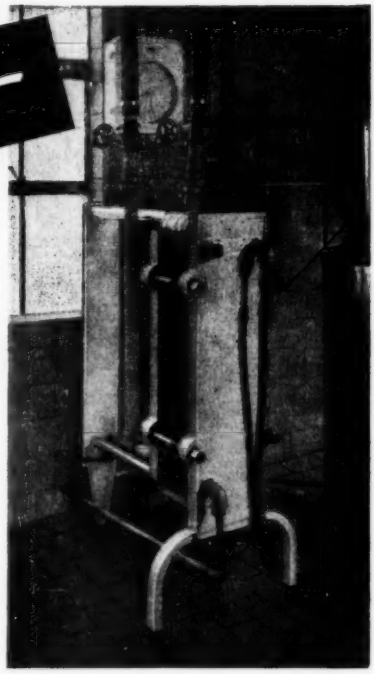

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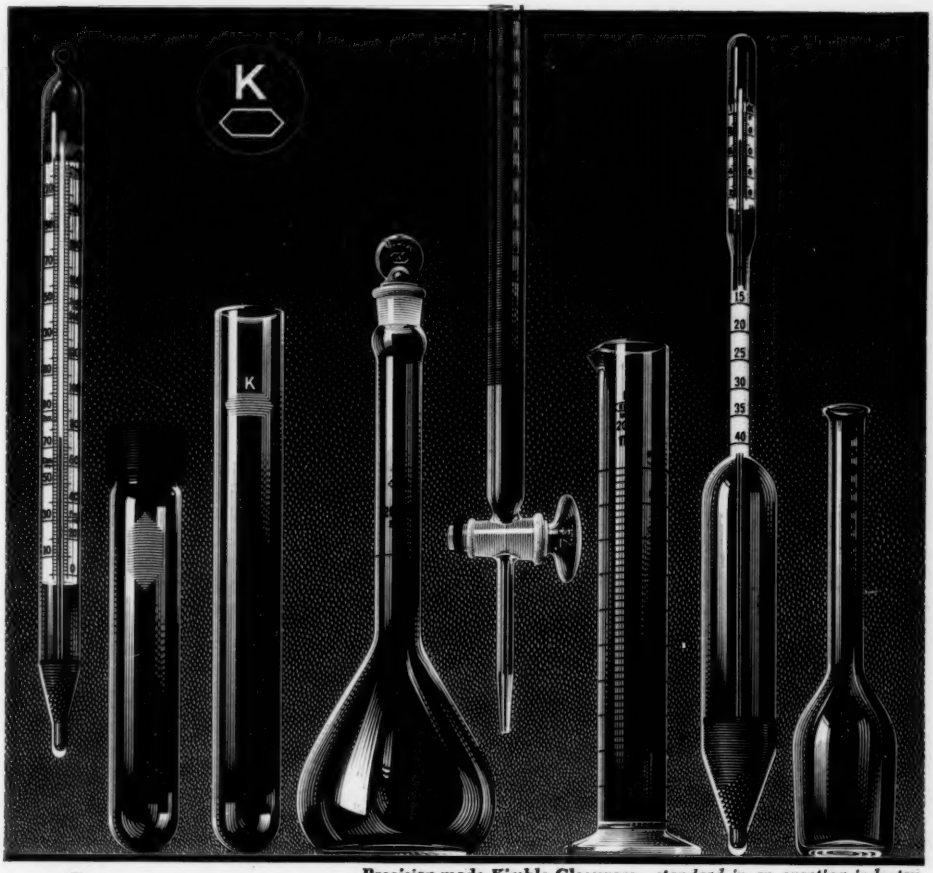

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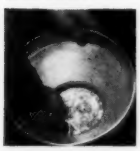

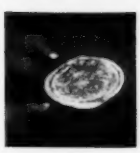

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JOURNAL OF DAIRY SCIENCE

VOLUME XXXVII June, 1954 NUMBER 6

ABSTRACTS OF PAPERS PRESENTED AT THE FORTY-NINTH ANNUAL MEETING

The Pennsylvania State University
State College
June 20-24, 1954

MANUFACTURING SECTION

M1. Physico-chemical changes induced in casein by Streptococcus agalactiae. G. W. MALANEY, H. H. WEISER, and Q. VAN WINKLE, The Ohio State Univ., Columbus.

A study of the physico-chemical alterations produced by *Streptococcus agalactiae* in the casein of cow's milk showed that the microorganism is able to induce a reduction in solubility and a change in the relative concentrations of the electrophoretic components of casein.

The latter change revealed itself as an increase in the relative concentration of β- or γ-casein, accompanied by a proportionate decrease in the relative concentration of α- or β-casein, respectively. The values obtained for the distribution of free amino groups by formol titration indicated that the changes are undoubtedly not the result of protein hydrolysis.

It is suggested that the shift in the proportions of α-, β-, and γ-casein is a disaggregation reaction involving α-β, and β-γ complexes. It is probable that a 2nd factor is operative in this change; the poorly-soluble α-casein, once dissociated from the β-casein, precipitates to some extent, thus further increasing the relative concentration of β-casein.

The reduction in casein solubility apparently derives from denaturation of one or more components and the low solubility of α- and possibly γ-casein when dissociated from β-casein.

The experimental results agree closely with the theory advanced by Linderstrøm-Lang, who suggested that one of the components of casein must act as a protective colloid for the dispersion of calcium caseinate as it exists in milk. It is tentatively postulated by the authors that β-casein is Linderstrøm-Lang's protective colloid which maintains the components in solution in milk.

M2. Heat denaturation of the specific serum proteins in milk. B. L. LARSON and G. D. ROLLERI, Univ. of Illinois, Urbana.

Milk was heat treated at 8 temperatures between 56° and 96° C. for 30 min., and the proteins in the serum obtained after removal of the denatured serum proteins with the casein at pH 4.6 were examined electrophoretically. The total serum proteins (100%), the classical "globulin" fraction (13.0%, accounted for by the immune globulins), and the proteose-peptone fraction (18.9%) evaluated in the electrophoretic procedure agreed well with comparative analyses conducted according to the procedures of Rowland *(J. Dairy Research,* **9**: 42, 1938). In addition, the summation of the α-lactalbumin (19.7%), β-lactoglobulin (43.7%), and serum albumin (4.7%) contents accounted for the classical "lactalbumin" fraction (68.1%).

The denaturation curves obtained for each protein indicated that the immune globulins are the least and α-lactalbumin the most heat resistant of the milk serum proteins. The heat treatment of 70° C., for example, denatured 89% of the immune globulins, 52% of the serum albumin, 32% of the β-lactoglobulin, and 6% of the α-lactalbumin.

The proteins of the serum prepared from the milk heated at 96° C. corresponded to the proteose-peptone fraction of Rowland, and the 3 components identified in the electrophoretic pattern were present in the unheated and all of the heated milks at the same concentration.

M3. The effect of heat and salts on the centrifugal sedimentation rates of milk proteins. L. F. EDMONDSON and N. P. TARASSUK, Univ. of California, Davis.

Fresh skimmilk was subjected to centrifugal separation for successively increasing periods of time at 20° C. in a Spinco preparative centrifuge at 20,000 r.p.m. This is equivalent to an average force of 26,000 times gravity. The supernatant liquid was carefully removed and analyzed for total nitrogen, noncasein nitrogen, total calcium and phosphorus. The results showed a slight decrease in the rate of nitrogen sedimentation for milks heated to 88° C. for 15 min.

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when compared to the raw milk samples, which indicates a decrease in the size of the caseinate particles. A further decrease resulted when 0.5% and 0.15% Na_2HPO_4 was added to the heated samples. No change in the rate resulted when the Na_2HPO_4 was added to the raw skimmilk and centrifuged. Increasing rates of nitrogen sedimentation resulted when $CaCl_2$ was added to the raw milk. Experiments with centrifugally obtained whey, which was subsequently heated, showed that the heat-denatured whey proteins were removed by centrifugation, whereas they were unaffected in the raw milk. The calcium/nitrogen, and phosphorus/nitrogen ratios in the supernatant liquid were slightly less in the heated sample and distinctly lower in the Na_2HPO_4 treated samples than in the raw milk. The calcium/phosphorus ratio was the same for all samples regardless of centrifuging time.

M4. Sedimentation of milk proteins as a function of certain ions. S. J. Bishov and J. H. Mitchell, Jr., Quartermaster Food and Container Inst. for the Armed Forces, Chicago.

Studies of sediments of supercentrifuged calcium-altered fluid and spray-dried milk showed that a direct relationship exists between the calcium level and the quantity of sediment proteins. Additions of calcium to milk increased the quantity of sediment and complexed calcium over normal levels. This effect was reversed when calcium was removed by ion exchange or sequestered by a chelating agent. The substitution of equivalent quantities of sodium, potassium, or magnesium for calcium decreased the quantity of protein sediment. Addition of phosphate did not affect the sediment composition significantly, whereas the addition of citrate did. The optical density of milk decreased with calcium removal.

Reconstituted spray-dried milk yielded more sediment than fresh milk; however, by reduction of the calcium content of this product the quantity of sediment was reduced to the levels obtained with fresh milk. No separation of skimmilk was effected under a force of 36,000 $\times$ g applied for 15 min. when the calcium level was 1/3 of normal.

These investigations were made in connection with research on quality differences between fresh milk and reconstituted spray-dried milk.

M5. The activity of calcium chloride, citrate and dihydrogen phosphate in casein sols. H. E. Affsprung, C. W. Gehrke, and J. M. Baker, Univ. of Missouri, Columbia.

Solutions of the calcium salts of chloride, citrate and dihydrogen phosphate have been made in casein sols and the activities measured with ion-exchange resin membrane electrodes. The data are presented graphically, and the potential measurements obtained in casein sols are compared to those obtained in true solutions of the same salts at the same molar concentrations and to theoretical curves calculated from the Debye-Hückel theory. The various solutions, in casein sols, were heated at 120° C. for 15 min. and cooled, and the activities were remeasured. The pH was checked in nearly all solutions, heated and unheated. No interaction of the casein with the calcium, in either the unheated or the heated solutions could be found. It was noted that when the calcium ion activity reached a value of about 0.03 the casein sol coagulated, although no significant change occurred in the measured activity of the calcium or in the pH. The behavior of the casein in all the solutions resembled that of a hydrophobic colloid.

M6. Characterization of some lactobacilli[1] found in milk. J. E. Edmondson and R. G. Jensen, Univ. of Missouri, Columbia.

Lactobacilli were isolated by the enrichment method from 561 Grades A and C milk, pasteurized milk, and sour cream samples. Several isolation media were tried with plain tomato juice (V-8 juice) agar giving as good or better results than the others. Direct plating with Rogosa SL agar proved to be of value in some cases.

The gas-producing lactobacilli were detected by using 5% glucose-milk agar and arginine hydrolysis.

The nongas-producing organisms were identified by Tittsler's classification, which involves colonial morphology, growth at 16° and 45° C., ability to coagulate milk, amount of acidity produced in milk, growth in a defined medium, and fermentation of various carbohydrates. Determination of lactic acid rotation was omitted. Brigg's classification, which involves gas production in 5% glucose agar, hydrolysis of arginine to form NH_3, tolerance of 4% NaCl, growth at 15°, 45°, and 48° C. and survivals at 60° for 90 min. and 65° for 30 min., was applied to both the gas formers and nongas formers. In addition the ability of the gas formers to ferment a number of carbohydrates was checked.

Thirty-two known cultures were obtained from various sources and checked by both classifications.

[1] Series No. 1413. Approved by the Director.

M7. Stimulation of lactic acid bacteria by substances concomitant to certain proteolytic enzymes. D. E. Kizer, L. Hankin, M. L. Speck, and L. W. Aurand, N. C. State College, Raleigh.

It was observed that substances accompanying partially purified unhydrolyzed trypsin, pepsin, pancreatin, and erepsin exerted a pronounced stimulatory effect upon the growth and lactic acid production of *Lactobacillus casei* and *Streptococcus lactis*. These effects were observed in a synthetic medium (Roberts and Snell, *J. Biol. Chem.*, 163:499. 1946) and in milk. The substance(s) caused an increase in cell numbers, as measured turbidimetrically, and increased

acid production, as measured titrimetrically. The active factor(s) did not appear to be an integral part of the enzyme, since crystalline enzymes possessed no stimulatory effect. Studies on lactalbumin, hydrolyzed with partially purified trypsin (1-300 trypsin, Nutritional Biochemical Corp.), showed that the majority of the activity of such hydrolyzates was due to the factor(s) accompanying the enzyme rather than to products of protein cleavage. Further, preliminary studies indicated that a portion of the stimulation manifested by enzymatically hydrolyzed casein was due to the factor(s) associated with the partially purified enzymes. The site of mammalian tryptic enzyme formation, i.e., the pancreas, has been found to be a rich source of the active factor(s).

M8. Metabolism of amino acids by Lactobacillus casei. T. KRISTOFFERSEN and F. E. NELSON, Iowa State College, Ames.

Cells of *L. casei*, grown in a V-8 juice broth without added carbohydrates, were tested for their ability to deaminate amino acids. Of 18 amino acids and glutamine and asparagine only serine was deaminated at pH 5.4. At pH 8.1 asparagine and threonine also were deaminated, but the rate of deamination of serine was about 4 times greater. Histidine, tryptophan, arginine, and cysteine inhibited the endogenous reaction. Glutamine hydrolyzed spontaneously and the remaining amino acids were indifferent.

A further investigation of the serine deaminase of *L. casei* showed the optimum pH to be about 8.3 and the minimum about 4.0 with approximately half as much activity at pH 5.0-5.4 as at pH 8.3. Pyruvic and lactic acids have been demonstrated in the reaction mixture.

At 15 hr. old, culture of *L. casei* grown in above mentioned medium has a pronounced odor of H_2S. The odor first becomes apparent when pH of the culture gets below 4.8 and is very strong at harvest time at pH 4.3.

The role of deamination of serine and production of H_2S by *L. casei* in relation to flavor development in Cheddar cheese is being investigated. Samples of Cheddar cheese from different sources have all been shown to contain H_2S.

M9. Studies on corn steep liquor in the nutrition of lactic acid bacteria. H. E. KENNEDY, M. L. SPECK, and L. W. AURAND, N. C. State College, Raleigh.

The addition of 1% whole corn steep liquor to milk resulted in stimulation of growth of *S. lactis*, *S. cremoris*, *S. thermophilis*, *L. casei*, *L. arabinosus*, *L. bulgaricus*, *Leuc. citrovorum*, *Leuc. dextranicum*, and *Prop. shermanii*. Its effect on *E. coli* was questionable, and *B. subtilis* and *Ps. aeruginosa* were not stimulated. *S. lactis*, *L. casei*, and *L. bulgaricus* were stimulated by the addition of corn steep liquor to reportedly complete synthetic media for these organisms. Indications are that fermentation periods may be shortened by the use of the corn steep factor in the preparation of fermented dairy products.

Quantitative assay techniques using *L. casei* and *S. lactis* in milk and *L. casei* in synthetic media have been developed. Stability and solubility properties indicate that the factor cannot be associated with any of the reported growth stimulants for lactic acid bacteria. Also, the response of *L. casei* which is elicited by the factor is not replaceable in synthetic media by any of the reported stimulants.

M10. Acetate-citrate metabolism of Streptococcus lactis and Streptococcus cremoris. D. E. KIZER and M. L. SPECK, N. C. State College, Raleigh.

A completely synthetic medium (Henderson and Snell, *J. Biol. Chem.*, **172**: 15. 1948) containing either 10 mg/ml of citrate, 10 mg/ml of acetate or none of either was used to study the acetate-citrate metabolism of *S. lactis* and *S. cremoris*. Cells were grown in litmus milk and inoculated into the test media at a rate of 0.05% and incubated for a period of 24 hr. at 32° C. This was followed by 2 serial transfers in the test media with identical inoculation rates, incubation times, and temperature; growth was determined turbidimetrically. The strains were placed in two groups for statistical analysis on the basis of their reactions in maltose medium and the presence or absence of growth at 40° C.

Statistical analysis of the data showed that the group of organisms that utilized maltose and grew at 40° C. responded significantly to citrate; they failed to show a significant response to acetate. The group of organisms that failed to utilize maltose and did not grow at 40° C., gave a significant response to acetate, but did not respond significantly to citrate. The application of these results to problems in the classification in the lactic group of streptococci will be discussed.

M11. Changes in the bacterial population of milk due to the addition of some chemical bactericides. F. J. BABEL, Purdue Univ., Lafayette, Ind.

Various concentrations of hypochlorite, chloramine-T, 1,3-dichloro-5,5-dimethyl hydantoin, and an iodine bactericide were added to raw milk and the bacterial population determined initially and after 24 and 48 hr. A concentration of approximately 100 p.p.m. available chlorine added as hypochlorite, chloramine-T or 1,3-dichloro-5,5-dimethyl hydantoin was required to prevent an increase in bacterial population of raw milk when the milk was held for 24 hr. at 40° F. A concentration of approximately 200 p.p.m. available chlorine added as hypochlorite and 500 p.p.m. available chlorine added as chloramine-T or 1,3-dichloro-5,5-dimethyl hydantoin was required to prevent an increase in bacterial population when the milk

was held for 48 hr. at 40° F. An iodine bactericide in a concentration of 40 p.p.m. prevented growth of bacteria in raw milk held for 24 hr., and 40 to 100 p.p.m. prevented growth for 48 hr. at 40° F. With all of the chlorine compounds the addition of 200 to 500 p.p.m. available chlorine to raw milk was necessary to cause an appreciable decrease in bacterial population.

Acid production by lactic cultures was slightly inhibited by addition of 5 p.p.m. available chlorine to milk and greatly inhibited by 25 p.p.m. added as hypochlorite. Iodine bactericide added to milk in amounts of 5 to 50 p.p.m. had no effect on acid production by lactic cultures; small quantities of the bactericide actually stimulated acid production.

M12. Thermal death time studies of Brucella abortus in milk. F. R. KRONENWETT, S. A. LEAR, and H. J. METZGER, Rutgers Univ., New Brunswick, N. J.

The rate of heat inactivation of 8 strains of *Brucella abortus* (19, 659, 2016, 2308, 3237, McComb, Thompson Woodward) in milk was determined over the temperature range of approximately 61 to 68° C. The age of the cultures was known and concentrations were determined by the plate count. Suspensions of the organisms were automatically injected into 1,500 ml. of milk at predetermined temperatures. Samples were automatically taken in containers resting in an ice water bath.

The results are expressed as the slope of a straight line curve over one log cycle on semilogarithmic paper. The experimental range for Z values of 8 strains of Brucella abortus in this study is 4.3° to 4.8° C.

The age of the culture and concentration of cells did not affect the Z value of the organisms studied.

The present pasteurization standards of 143° F. for 30 min. and 161° F. for 15 sec. afford a margin of safety of approximately 26 min. and 12 sec., respectively.

M13. The effect of some factors on the growth of "psychrophilic" bacteria. W. C. LAWTON and F. E. NELSON, Iowa State College, Ames.

Because of the importance being attached to "psychrophilic" bacteria at this time it was considered desirable to study the effect of various factors on the growth of pure cultures when they were stored in milk at various temperatures. Since the size of the inoculum is of primary practical importance, samples were inoculated with varying amounts of a pure culture and stored at 5 and 25° C. At both temperatures the growth curves rose parallel to one another for the 3 sizes of inocula used and reached essentially the same final count.

The effect of partial heat destruction on the subsequent growth curves of organisms is of considerable practical importance. Cultures were partially inactivated by heat and inoculated into sterile skimmilk for storage at 5, 10, and 25° C. Varying concentrations of inocula were used for each temperature. The growth curves obtained were considerably altered by the action of heat. At 5 and 10° C. the lag period was considerably extended and in some cases viable organisms were not demonstrated after prolonged storage, although they were present in the early period of storage. At 25° C. the lag phase was extended but growth was very rapid during the logarithmic phase. The effects of chlorine also are being studied in the same manner and the results will be reported.

M14. A study of coliforms isolated from pasteurized milk. W. G. WALTER and L. T. CHRISTIAN, Montana State College, Bozeman.

Routine bacteriological tests on retail pasteurized milk and cream sometimes resulted in low standard plate counts of generally less than 10,000 organisms per ml. but high coliform counts ranging from 10 to several hundred per ml. In such instances representative coliform colonies were picked from desoxycholate lactose agar and stored at 5° C. on nutrient agar slants. Approximately 150 strains of coliform organisms were isolated over a period of a year and identified on the basis of their IMViC reactions, motility, gelatin liquefaction, and hydrogen sulfide production.

The majority of the strains appear to be intermediates between *Escherichia coli* and *Aerobacter aerogenes*. In most instances, high coliform counts could be traced to unsanitary practices or improperly cleaned equipment between the pasteurizer and the filler.

M15. Bacteriological studies of milk from menadione fed cows. H. H. WILKOWSKE, W. A. KRIENKE, L. R. ARRINGTON, and E. L. FOUTS, Univ. of Florida, Gainesville.

Menadione (2-methyl-naphthoquinone) was fed to dairy cows by mixing it into the concentrate of the ration. Each of 4 cows received 25 mg. daily for 28 d., 2 cows 100 mg. daily for 20 d., and 4 cows 600 mg. daily for 20 d.

Samples of milk were collected and subjected to bacteriological studies. One portion of each sample was incubated at 30° C. and titratable acidity determinations were made periodically to determine the rate of acid development by the normal contaminating flora of the raw milk. Second portions were inoculated with various commercial buttermilk and cheese cultures and treated as the first portion. Other portions were subjected to the standard coliform and psychrophilic plate counts at intervals during 4 wk. storage at 5° C.

Under feeding conditions as practiced in these studies, the feeding of menadione did not affect the normal rate of milk souring, the rate of lactic acid development by dairy cultures, or the standard coliform and psychrophilic plate counts.

Preliminary observations indicated that when

menadione was added directly to skimmilk at the rate of 10 γ per milliliter (some question whether all dissolved), there was a reduction in the rate of lactic acid development when commercial dairy starters were propagated in the treated milk.

M16. The use of a lyophilized lactic starter in the preparation of cottage cheese and cultured milk. J. C. WATTS and H. E. CALBERT, Univ. of Wisconsin, Madison.

A frozen-dried preparation of lactic starter has been developed which shows unusual activity in the production of acid, flavor, and aroma. Cottage cheese and cultured milks were made by direct inoculation of this lyophilized starter. The level of inoculum required for cottage cheese is 5 to 7 g. per 100 lb. of milk. Two to 3 g. added to the same amount of milk produced excellent cultured milk.

The lyophilized culture can be stored for varying periods of time with only slight loss of activity. This depends on the temperature of storage. Starter cultures prepared in this manner exhibited satisfactory activity after storage of 2 wk. at 70-80° F., 4 wk. at 38° F., and 14 wk. at –20° F.

Packaging of the preparation in vacuo or under nitrogen holds promise of extending shelf life of the product at temperatures above freezing.

M17. A study of some factors affecting the quality of cottage cheese. M. P. RHODES and L. G. HARMON, Texas Technological College, Lubbock.

Samples of commercial and eperimental creamed cottage cheese were incubated at 50° F. and analyzed at 0, 3, and 7 d. for free tyrosine, free fatty acids, and pH. Also, proteolytic, lipolytic, coliform, and yeast and mold counts were made at each sampling interval.

The initial free tyrosine content of 12 commercial samples ranged from 91 to 136 p.p.m. and after 7 d. at 50° F. increased to a range of 123 to 273 p.p.m. Eight experimental samples had initial values of 52 to 73 p.p.m. and after 7 d. increased to 85 to 95 p.p.m. Some relationship existed between free tyrosine content and occurrence of bitter, putrid, or decomposed flavor, with off-flavors appearing when free tyrosine reached 125 to 150 p.p.m.

Free fatty acid values are expressed as the ml. of 0.1 *N* KOH required to neutralize 10 g. of fat. Initial values for 12 commercial samples ranged from 0.75 to 2.78 and after 7 d. had increased to 1.81 to 11.52. Eight experimental samples had initial values ranging from 1.76 to 2.74, and values after 7 d. ranged from 1.65 to 2.2.

Many of the commercial samples exhibited a rancid flavor after 7 d., but very little relationship was observed between free fatty acid value and rancid flavor or odor.

M18. The effect of fortification of fluid skimmilk with nonfat dry milk solids on the yield of cottage cheese curd. R. W. MYKLEBY and B. M. ZAKARIASEN, Land O' Lakes Creameries, Inc., Minneapolis.

The fortification of fluid skimmilk with low heat nonfat dry milk solids for cottage cheese making resulted in yields of 2.29 to 2.38 lb. of curd per lb. of nonfat dry milk solids used. This compared to yields of 1.42 to 1.45 lb. of cottage cheese curd per lb. of nonfat solids when only the fluid skimmilk was used.

From the time of cutting throughout the entire cooking process, the cheese curd from the fortified vats was easier to handle, could stand more abuse, and was more uniform in size than the curd from the unfortified vats. Acid development was slightly faster, the cooking period was generally shorter, and the size of curd particles was larger with increasing degrees of fortification.

Fluid skimmilk with 8.7% T.S. is at a critically low solids level for the manufacture of cottage cheese curd having good body, texture, and handling qualities. The fortification of this milk with added nonfat solids predisposes it to better curd-forming qualities.

M19. Influence of the bulk handling of raw milk on its rennet coagulation time. HAROLD RAPP and H. E. CALBERT, Univ. of Wisconsin, Madison.

The bulk method of handling milk on the farm requires holding the milk for periods of several days at temperatures of 40° F., or below. The effect of this cool aging treatment on the rennet coagulation time of the raw milk was studied.

Cool aging of raw milk decreases the susceptibility of the milk to rennet coagulation. This effect, although variable in magnitude in mixed milk samples, was observed in all of the samples studied. The average increase in the rennet coagulation time of mixed night's and morning's milk held at 36° F. was 4% after 1 d. holding, 7.2% after 2 d. holding, and 12.5% after 3 d. holding.

It was observed that the period of time during which the milk samples were held in the tempering water bath after they had reached 30° C. had a great influence on the rennet coagulation time of the milk. To obtain comparable results in studying the susceptibility of milk to coagulation, the tempering time of the samples must be standardized.

M20. Flavor development in pasteurized milk Cheddar cheese. I. The effect of inoculating milk with Geotrichum candidum. O. R. IRVINE, D. H. BULLOCK, and W. H. SPROULE, Ontario Agr. College, Guelph.

Both water-soluble and water-insoluble volatile acids, as well as cheese fat acidities, were found to increase to a much greater extent during curing in factory made, raw milk Cheddar

cheese than did these values in laboratory made, pasteurized milk cheese. A strain of *Geotrichum candidum*, isolated from raw milk curd, was found to promote a similar pattern of fatty acid liberation to that of raw milk cheese when inoculated into pasteurized milk 18 hr. prior to cheesemaking. In most cases the inoculated cheese received equal or higher flavor scores than the controls and appeared more mature. Inoculations did not result in rapid development of sharp "cheese" flavor, however. Inoculated milk previously pasteurized and homogenized, yielded rancid flavored cheese with very high volatile acid values and cheese fat acidities.

Silicic acid column chromatographic determinations made on inoculated and uninoculated pasteurized milk cheese as well as on factory made raw milk cheese indicated that the inoculated and raw milk cheese chromatograms were similar, both showing the presence of significant amounts of C_6 and higher acids. These acids were almost completely absent in the uninoculated controls.

M21. Further studies on the use of ultrasonic waves to accelerate the ripening rate of cheese. J. H. Nelson and W. C. Winder, Univ. of Wisconsin, Madison.

Previous research demonstrated that ultrasonic waves accelerated the ripening rate of Cheddar cheese. The present investigation was made to develop ultrasonic equipment which could be used to treat cheese on a pilot plant scale, and to establish treatment conditions which would produce maximum acceleration of ripening. Serious limitations in quartz piezoelectric elements which were employed in the original research stimulated the development of equipment which utilized barium titanate-ceramic piezoelectric elements. The use of 2-stage generators overcame unstable operation caused by the low electrical impedance of the titanate elements.

Cheese samples were treated by placing them in physical contact with barium titanate elements. This method was more efficient and practical than methods in which the acoustic energy was transmitted to samples through one or more media. Optimum treatment conditions were determined empirically at low acoustic energy levels. Ripening rate acceleration produced by titanate elements at these energy levels was less than that produced by quartz elements. More powerful equipment recently developed may prove titanate and quartz equally effective. Utilization of new methods which measure acoustic energy directly will enable fundamental evaluation of treatment conditions. Studies on mechanisms of ultrasonic effects in cheese indicate that the biological systems are most affected.

M22. Effects of temperature and pH on the survival and growth of Swiss cheese bacteria. R. P. Tittsler, J. Wolk, and R. E. Hargrove, Washington Utilization Research Branch, U. S. D. A.

Studies were made to establish the optimum temperatures and pH levels for the survival and growth of Swiss cheese bacteria during the cooking and pressing of the curd. The bacteria were heated in milk at different temperatures and pH levels. The temperature was *(a)* increased and then decreased gradually to reproduce conditions occurring in the manufacture of Swiss cheese; *(b)* held constant throughout the experiment; *(c)* held constant for a time, then lowered quickly to 48 or 46° C. and either maintained or allowed to drop gradually for several hours. Critical temperatures for survival and favorable temperatures for growth of the bacteria at different pH levels have been fairly well established.

The results emphasize the effects of temperature and pH during cooking and pressing Swiss cheese curd on the survival and subsequent growth of the starter bacteria. They show that the temperature during pressing the curd is much more important than that during cooking. Furthermore, they indicate that an important function of the *Streptococcus thermophilus* starter is to produce sufficient acid early in the pressing stage so that *Lactobacillus bulgaricus* or *Lactobacillus lactis* can survive and subsequently grow at the temperature of the curd during pressing.

M23. Factors affecting the organic acid production of propionbacteria used in the manufacture of Swiss cheese.[1] I. S. El-Hagarawy, W. L. Slatter, W. J. Harper, and I. A. Gould, The Ohio State Univ., Columbus.

This study was made to obtain basic information regarding the factors influencing the production of propionic and acetic acids by *P. shermanii*. There was little variation in the maximum amounts of the acids produced by 9 of 10 strains of *P. shermanii* in broth media during a 12-d. period.

The incorporation of a 1% sodium lactate in broth media resulted in a more rapid rate of acid production than occurred when lactose was used in the same concentration. Lactate added to milk materially increased the propionic and acetic acid production, the concentrations formed being equal or higher than that produced in broth media. Other factors increasing the acid production in milk media were yeast extract and protein hydrolyzates.

The addition of 0.5% yeast extract to a reconstituted milk medium increased the production of acid from 2.25 to 4.5 fold in comparison to that produced in the milk control after 4 d. of incubation. The addition of 0.1% peptonized milk or 1% casein hydrolyzate to the milk medium resulted in increases up to 350% in acid

[1] Supported in part by the Ohio Dairy Products Research Fund.

production. The addition of biotin, thiamine, pantothenic acid and *P. aminobenzoic* acid to the milk medium had no effect on acid production.

M24. Free amino and fatty acids in commercial Swiss cheese.[1] P. C. HINTZ, W. L. SLATTER, and W. J. HARPER, The Ohio State Univ., Columbus.

Ion exchange chromatography was used to analyze 13 aged Swiss cheeses for 14 amino acids. The same cheeses were also analyzed by chromatography for higher fatty acids and for valeric, butyric, and propionic acids. Not all acids were present in all the cheese, and there was a wide variation in concentration of different acids among the cheeses.

Amounts of cysteic acid, taurine, proline, lysine, and histidine tended to increase with the age of the cheese, whereas amounts of aspartic acid and glycine remained relatively constant. The concentrations of threonine-serine, asparagine, glutamic acid, tyrosine-phenylalanine, and tryptophane decreased as ripening progressed.

Butyric, propionic and higher acids were present in all cheeses studied, and valeric acid was present in all but one cheese. There was no relationship betwen age and the amounts of these acids present.

A minimum proline concentration of 12.00 mg/g and a minimum propionic acid concentration of 9.00 mg/g both were necessary before a cheese was found to exhibit a medium intensity of characteristic flavor.

High concentrations of histidine were found in cheeses having low flavor ratings, but it appeared that the deleterious effects of an excess of this acid could be overcome by other factors.

[1] Supported in part by the Ohio Dairy Products Research Fund.

M25. A low-fat soft-ripened cheese.[1] R. A. REISFELD and W. J. HARPER, The Ohio State Univ., Columbus.

A new low-fat cheese was made from a milk–sweet cream–buttermilk mixture containing 2.0% fat and 10-30% buttermilk. The use of 10% buttermilk resulted in a brick cheese type body, whereas the use of 30% buttermilk resulted in cheese with a very soft, spreadable body. Fluid, condensed, and dried buttermilk have been used successfully. A modification of the rapid brick cheese manufacturing method of Buyens and Price (1952) was selected since it required a minimum of time and special equipment.

Parallel increases in moisture and salt content of the cheese appear to be related to change in curd structure caused by the buttermilk. Cheeses which may be cured between 40°-60° F. show no noticeable difference in the rate of body breakdown; however, less texture defects develop at the lower temperature. The cheese is salable after 6 wk. of ripening and has a shelf life of approximately 6 mo. The average composition of the cheese is 25% fat on dry weight basis, 44-46% moisture, 1.5-1.7% salt.

Limited consumer trials have shown good acceptance of this low-fat product, especially the soft, spreadable cheese.

[1] Supported in part by the Ohio Dairy Products Research Fund.

M26. The heat processing of Italian curd into Mozzarelle cheese. F. V. KOSIKOWSKY and G. J. SILVERMAN, Cornell Univ., Ithaca, N. Y.

Mozzarelle cheese made by conventional time-consuming methods has a relatively short shelf life. Processing the ripened curd directly in steam-jacketed cheese cookers and eliminating the tedious stretching and molding of the curd in hot water, if successful, might result in increased operational efficiencies and a product having better keeping qualities.

Eighteen vats of whole milk were made into curd and ripened from 2 hr. to 4 d. Five-lb. and 100-lb. lots of this curd, in addition to a series of commercial lots, were heated in process cheese cookers. Heating properly ripened Italian curd to 160° F., using 2-3% sodium citrate, resulted in a smooth plastic mass. With certain lots of cheese adequate emulsification was obtained at 0.5% levels of sodium citrate, but it was not possible to eliminate processing salts entirely.

When cooled, the resulting product, depending upon past treatment, ranged in pH from 5.3 to 6.2 and the moisture from 38 to 58%. Physically it resembled Mozzarelle cheese, and critical appraisals by experienced manufacturers indicated excellent flavor and cutting qualities. One criticism expressed was that this cheese did not "string out" quite as well upon application to Pizza pie. This would not necessarily deter consumer acceptance.

Processing operations are very critical. Rapid removal of the hot mass from the cooker is necessary to avoid a resulting toughening and translucency of the cheese. Flowing ability is considerably lower than that of processed Cheddar cheese and may present problems in filling operations.

M27. A study of lyophilization as a means of preserving lactic starters. J. C. WATTS and H. E. CALBERT, Univ. of Wisconsin, Madison.

This study was undertaken to investigate the process of lyophilization as a means of preparing active lactic starters. The influence of the following factors on this process was considered: strains of incorporated organisms, age of culture, titratable acidity, per cent inoculum, freeze-drying medium, and repropagation and refreeze-drying.

Cultures were evaluated by comparing acid, flavor, and aroma production before and after lyophilization. The Horrall-Elliker and a modi-

fied creatine test were applied in these determinations.

The optimum age range was shown to include the late log and early maximum stationary phases. Titratable acidities of 0.80% or lower did not affect abilities after freeze-drying. This was demonstrated by comparing neutralized and nonneutralized preparations. Inocula of 1-15% produced cultures of equal capabilities. Ten% reconstituted skimmilk was utilized as a satisfactory lyophilization medium. Repropagation and refreeze-drying caused a slight increase in activity.

M28. The effect of emulsifier on growth and toxin production of Clostridium botulinum experimentally inoculated into surface-ripened cheese. R. O. WAGENAAR and G. M. DACK, Univ. of Chicago.

In the manufacture of cheese spreads an emulsifier is added to keep the fat uniformly distributed. The emulsifiers most commonly used are disodium phosphate and sodium citrate. In the experiments to be discussed toxin-free spores of type A *Clostridium botulinum* were inoculated into cheese preparations both with and without added emulsifier. The tubed samples were transferred to a desiccator jar which was evacuated and anaerobiosis established with sodium pyrogallate solution in an atmosphere of 10% CO_2. Incubation was at 30° C. Samples were removed for toxin and pH analyses at appropriate intervals. Growth and toxin production of *Cl. botulinum* is affected by the NaCl concentration in cheese spread. It was found that the addition of 2.5% disodium phosphate reduced the amount of NaCl required for inhibition. This inhibition was equivalent to the effect produced by increasing the NaCl level in the cheese approximately 1.0%. Similar results were obtained when 2.5% sodium citrate was tested, although this salt seemed to be less inhibitory than disodium phosphate. Experiments with disodium phosphate at the 1.25% level showed that the lesser quantity of this emulsifier was only slightly inhibitory when compared to cheese samples with no emulsifier added.

M29. Effect of certain germicides in sanitizing teat cup inflations of milking machines. W. T. EZZARD and F. W. BENNETT, Univ. of Georgia, Athens.

The survival of bacteria in milking machine teat cup inflations after treatment with solutions of certain sanitizing agents was studied. The teat cups were dipped in tap water, solutions of hypochlorites, or a quaternary ammonium compound (alkyldimethylbenzylammonium chloride) after each cow was milked. Rinse test counts of bacteria were made on the inflations at intervals during the milking of the herd. The declines in the strengths of the hypochlorite solutions during usage were measured. Rinse tests were made also after the filling of the inflations with solutions of lye or hypochlorites and storage on racks between milkings.

Lower rinse test counts were obtained when the teat cups were dipped momentarily in hypochlorite solutions instead of tap water. Further reductions were obtained when the time of exposure was extended to 2 min. The use of solutions of quaternary ammonium compound resulted in lower counts than when hypochlorite solutions were used in momentary dipping, for the concentrations employed. The results were more nearly equal between the 2 kinds of solutions after 2-min. exposures. The strengths of the hypochlorite solutions declined as much as 85% during the milking of the herd.

Slightly lower rinse test counts of the inflations were obtained from the use of hypochlorite solutions than when lye solutions were the sanitizing agents in storing the cups between milkings.

M30. Laboratory study of milkstone formation. THADDEUS LEWANDOWSKI, Pennsylvania Salt Mfg. Co., Wyndmoor, Pa.

By means of the "Autotechnicon," weighed stainless steel strips were dipped consecutively into 200 p.p.m. available chlorine solutions, raw milk, water, cleaner, and water; this basic cycle was repeated 30 times. Rinses and solutions were composed of 300 p.p.m. hardness synthetic water. After solvent extraction, milkstone was determined by weight difference. Replacement of basic cycle components with hard water, or substituting distilled for hard water, indicated that all components were interrelated in respect to milkstone formation. A nonlinear relationship was demonstrated between water hardness and amount of milkstone formed. Use of a 120° F. first rinse resulted in almost double the milkstone produced with a room temperature rinse. Drying before the first rinse did not increase, whereas drying after the first rinse slightly decreased, milkstone deposition. There was little difference in milkstone formation between use of calcium hypochlorite, sodium hypochlorite, or a chlorinated orthophosphate. Use of a cationic detergent as a cleaner resulted in over twice the milkstone formed with an anionic or nonionic detergent. Milkstone was markedly reduced by use of polyphosphate, nonionic detergent, or acid cleaners. Cleaners containing hard water precipitating salts and sodium pyrophosphate produced the largest amounts of milkstone.

M31. Susceptibility of milk to copper-induced oxidized flavor and an inhibitory effect of menadione when included in the ration. W. A. KRIENKE and L. R. ARRINGTON, Univ. of Florida, Gainesville.

Menadione (2-methyl napthoquinone) was included in the concentrate portion of the rations of several producing cows at levels of 25 and 600 mg. per day. Samples of milk were collected at the evening milking, cooled, and stored under refrigeration until the following morning.

Copper, in the form of copper sulfate in solution, was added to different portions at levels of 1.5, 3.0, and 6.0 p.p.m. Each series included a control sample. Organoleptic observations were made after 2, 4 and 6 d. of refrigerated storage. The milks of several cows (regular ration including pasture and/or silage) and of 8 others (experimental ration free of succulent feeds) were found to vary individually in susceptibility to copper-induced oxidized flavor. The flavor defect of some samples was of the "fishy" rather than "cardboard" type. Susceptibility was greatly increased by the nonsuccelent ration. Menadione in the ration was effective to different degrees in lowering susceptibility. The addition and then removal of menadione in the ration of a few cows for 2 successive cycles was followed within a few days by decreases and increases, respectively, in susceptibility.

M32. The effect of certain treatments on the stability of ascorbic acid in milk. J. W. STULL and R. N. DAVIS, Univ. of Arizona, Tucson.

The effect of batch pasteurization, homogenization, surface cooling, exposure to sunlight and storage at 40° F. on natural and added ascorbic acid was observed in milk with varying concentrations of added copper, antioxidants, synergists, and chelating compounds. Antioxidants studied were nordihydroguaiaretic acid (NDGA) and 6-ethoxy-2,2,4-trimethyl-1,2- dihydroquinoline (Santoquin). Synergists, such as citric acid and methionine, and the chelating compounds of the ethylene diamine tetra acetic acid (EDTA) type were used.

Results indicate that combinations of from 50 to 100 p.p.m. of the antioxidants, synergists, and chelating compounds were effective in retarding the destruction of ascorbic acid in milk. Retardation was most effective during storage at 40° F. and least effective during exposure to sunlight. Development of oxidized and activated flavor was retarded by the addition of these materials. Citric acid exhibited a synergistic effect, as did added ascorbic acid. Nontypical flavor development was observed in the milk to which Santoquin was added. It was not possible to correlate ascorbic acid destruction with intensity or rate of development of off-flavor.

M33. Effect of ascorbic acid, amber bottles, daylight, homogenization, and pasteurization on the oxidized flavors of milk. E. S. GUTHRIE, Cornell Univ., Ithaca, N. Y.

When the ascorbic acid was quickly and completely oxidized from the milk before exposure to daylight in white bottles the oxidized flavors did not appear in the unhomogenized samples, and they were only slight in the homogenized ones. In the amber bottles the protection was complete after exposure to daylight in both the unhomogenized and homogenized milks.

In the check samples the milks showed oxidized flavors in both white and amber bottles and when both unhomogenized and homogenized. These flavors, however, were distinctly more pronounced in the white bottles and in the homogenized milks.

When ascorbic acid was added up to 100 mg/l of milk, highly oxidized flavors were developed in both the unhomogenized and homogenized samples after they were exposed to daylight. The protection by the amber bottles was great but not complete.

The oxidized flavors developed more readily in the milks that were exposed to daylight after pasteurization than in those exposed before pasteurization.

M34. The susceptibility of milk depleted of the total vitamin C content to oxidized flavors, as influenced by the roughages fed to the cow. V. N. KRUKOVSKY, Cornell Univ., Ithaca, N. Y.

The observations indicate that vitamin C depleted milk, upon the readdition of ascorbic acid and copper, does not necessarily undergo organoleptically detectable oxidative changes more readily than the natural milk. Its susceptibility to deterioration is governed by the presence and oxidizability of unknown substances in the watery phase of the milk, named non-flavor-forming compounds. The excretion of these compounds by the mammary gland is influenced by the roughages fed to the cow and the physiological response of the animal to feed. The addition of ascorbic acid and Cu (20 and 0.1 mg/l, resp.) to milk depleted of the total vitamin C content by H_2O_2 (0.03 ml. of 30% H_2O_2/l) and heat, or photochemically, but which was produced on orchard grass pasture, early corn silage and early barn-cured hay (predominantly timothy), failed to stimulate the oxidized flavors, whereas milk produced on ladino pasture, when treated the same way, developed flavors at a much faster rate than the natural milk. The organoleptic tests made on 233 samples revealed further that only the milk which underwent flavor changes during storage, with or without added copper as a catalyst, became more susceptible to deterioration after H_2O_2 or photochemical treatments.

M35. The buffer capacities of skimmilk, buttermilk, and butter serum from the same whole milk source. V. H. NIELSEN, E. W. BIRD, and PATRICIA CHANDLEE, Iowa State College, Ames.

Skimmilk, buttermilk, and butter serum were obtained from the same whole milk by separating the milk, churning the cream, and melting the butter at 45-50° C. To 5-ml. samples of these products were added decreasing amounts of 0.4 *N* HCl and increasing amounts of 0.4 *N* NaOH and enough distilled water to make the final volume of each sample equal to 6.1 ml. After 15 min. the pH of each sample was measured; the pH values obtained ranged from 1.75 to 11.75. The resulting titration curves indicate that of the 3 products, skimmilk has the highest

buffer effect and butter serum the lowest; this is particularly true in the range pH 2.0 to 7.0. The titration curves have the same general shape and tend to cross each other at approximately pH 6.25 in each experiment run.

Buffer indices (dB/dpH) were estimated from the titration curves for pH intervals of 0.50 unit. The buffer index values were plotted against the mean pH values at 0.25 pH intervals. The resulting curves indicate the presence in skimmilk of certain compounds or complexes (probably between calcium caseinate and calcium phosphate) which contribute buffer maxima at approximately pH 5.25-5.75 and pH 6.75-7.25. There is much less evidence of these complexes in buttermilk and butter serum. The results substantiate previous evidence to the effect that a calcium caseinate–calcium phosphate complex in butter serum is nonexisting or of little consequence.

M36. A method for measuring the efficiency of labor utilization in market milk plants. J. T. LAZAR, JR., and W. M. ROBERTS, N. C. State College, Raleigh.

A method for measuring the efficiency of labor utilization in fluid milk plants was developed. Observations were made of current ways of performing such jobs as bottle washing, case washing, bottle filling, stacking in cold storage, H.T.S.T. pasteurizing, homogenizing, separating, surging, handling sanitary pipe, and making cultured milk and special milk in many plants. The methods that were found were evaluated and improvements were made. Then standard times were established for the improved methods by using stop-watch and method-time measurement techniques. The efficiency of 2 dairy plants was determined by comparing the time in man-min. that was actually required to perform the processing of fluid milk products with the standard time that was calculated from the standard data.

M37. A plant study of bulk milk dispenser operations. E. J. FINNEGAN, E. M. BABB, JR., and G. C. WASSUM, Virginia Polytechnic Institute, Blacksburg.

Preliminary experiments have been conducted to determine the effect of several cleaning and filling procedures for 5-gal. milk dispenser cans on the sanitary and economic aspects of milk plant operations. Several types of cans were placed in operation in the Virginia Polytechnic Institute dairy plant for the bulk handling of pasteurized milk and chocolate milk. Time-motion and bacteriological studies were made to evaluate dispenser can operations in comparison to conventional milk bottle operations.

The average time to receive and hand scrub 1 dispenser can was 1.288 min., and the average time to machine wash and assemble 1 dispenser can was 0.681 min. The total man-hr. required to bottle 5 gal. of milk in ½ pt. bottles was 23.96 min., whereas the total man-hr. required to bottle 5 gal. of milk in dispenser cans was 14.29 min. The dispenser can filling device was a modification of a conventional terminal automatic can filler, wherein a float controls a feeding valve arrangement.

A bacteriological survey of the several aspects of dispenser can operations showed that the cleaning and filling procedures afforded a sanitary product which met the requirements of grade A milk.

M38. The viscosity of milk at selected temperatures as affected by homogenization pressure and type of homogenizer. C. H. WHITNAH, W. D. RUTZ, and H. C. FRYER, Kansas State College, Manhattan.

Parts of samples of freshly pasteurized milk were homogenized in a modified hand homogenizer at various pressures up to 250 p.s.i. Other parts of the same samples were similarly processed in a commercial Manton-Gaulin homogenizer at selected pressures from 200 to 3500 p.s.i. Viscosities of each of these parts were measured in an Ostwald type viscometer at temperatures of 4, 8, 18, 22.7, 29, 34, 39, and 44° C. Viscosities at these temperatures were also measured on samples of milk commercially homogenized at 2500 p.s.i. in a Cherry-Burrell viscolizer.

Small differences in viscosity could be consistently detected with this simple viscometer. The equation of Cox for the viscosity of milk relative to water at the same temperature and the equation of Cragoe for the viscosity of water at any specified temperature relative to its viscosity at 20° C. were used as a basis from which to measure deviations, due to processing, in the viscosity of milk.

Differences in viscosity due to the amount of pressure and type of homogenizer were much greater at a temperature of 4° C. than at 18° C. or higher. The 3 homogenizers appeared to give distinct families of curves relating to viscosity and temperature. A number of types of mathematical relationships between temperature and either relative or absolute viscosity of milk were studied. It appears that different equations may be required both for different types of homogenizers and for different pressure ranges for a given homogenizer.

M39. The identification of fat globules in the internal structure of ice cream. L. F. CREMERS and W. S. ARBUCKLE, Univ. of Maryland, College Park.

The method used by previous investigators for studying the internal structure of ice cream was used in this investigation. Thin sections of ice cream embedded in different mediums were examined with a regular microscope at a magnification of 430. If the embedding medium has the same or nearly the same refractive index as one of the ice cream materials, this particular material is not visible, but the other materials with a different refractive index can be seen.

Ice cream thin sections embedded in a mixture of amyl alcohol and kerosene, refractive index 1.430, show air cells, unfrozen material and ice crystals with refractive indices of 1.000, 1.460, and 1.310, respectively, but the fat globules, refractive index 1.430 to 1.450, are not visible.

Ethyl acetate and Acetone with refractive indices of 1.379 and 1.357, respectively, were used as immersion mediums to identify the fat globules in the internal structure of ice cream. Fat globules could be detected if dispersed in the unfrozen material and around the air cells, individually and in chain-like arrangement. There are indications that the fat globules orientate at least partly around the air cells, which indicates that there is a fat-air relationship. This fat-air relationship may vary with different percentage overrun and fat content and may influence the palatability of ice cream.

M40. Increasing solids-not-fat in ice cream. A process for the enzymatic hydrolysis of lactose. J. J. SAMPEY, Abbott's Dairies, Inc., Philadelphia, and C. E. NEUBECK, Rohm and Haas Co., Philadelphia.

The production of lactase from a lactose-fermenting yeast is described. Optimum activity of this enzyme occurs at 40° C. over the pH range 6.0-6.5. Under these conditions 1 part of lactase will hydrolize about 7 parts of lactose per hr. (in 10% lactose solutions). Hydrolysis proceeds more slowly after 40% of the lactose has been hydrolized. Data on the rate of lactose hydrolysis in condensed whole milk, condensed skimmilk, and sweetened condensed skimmilk are presented.

Concentrated skimmilk in which about 25% of the lactose has been hydrolized was used as a source of m.s.n.f. in the preparation of ice cream of 12% butterfat. At levels of 13-15% of m.s.n.f. these ice creams did not become sandy on cabinet storage which exceeded 4 mo., but controls with untreated m.s.n.f. showed the development of lactose crystals. Ice cream containing 13-15% of m.s.n.f. in which the lactose had been partially hydrolized showed the properties generally associated with higher levels of serum solids, such as increased body, smoothness, and stability.

M41. A study of the effect of heat upon ionic availability in milk. J. M. BAKER, C. W. GEHRKE, and H. E. AFFSPRUNG, Univ. of Missouri, Columbia.

An ion-exchange batch equilibration procedure is reviewed which has been used to compare the availability of calcium and phosphorus in raw and heat-treated skimmilk.

The heat treatments were made over a temperature range of 25° to 120° C. The period of heating was varied from 10 to 60 min. The process of heating was controlled to a ± 0.5° C. by constant temperature baths or an autoclave. A procedure was employed to prevent concentration by evaporation. The heat-treated samples were quickly cooled to room temperature and immediately equilibrated by the resin contact time procedure.

The data for the availability of calcium and phosphorus for exchange indicate that the ionic state is affected by more intense heats. The effect is more pronounced near the temperatures used for sterilizing some milk products.

The availability of calcium decreased in the range of 0-25% as the length and intensity of heating were increased. The effect upon phosphorus availability was not as pronounced.

M42. The use of aureomycin as a bacterial inhibitor for yeast and mold counts. H. C. OLSON, Oklahoma A. & M. College, Stillwater.

Yeasts and molds were found to be very resistant to the inhibitory action of certain antibiotics as compared to bacterial species commonly found in dairy products. A concentration of 100 p.p.m. of aureomycin hydrochloride added to potato dextrose agar just prior to pouring the plates effectively inhibited bacterial growth and apparently had little or no inhibitory effect on the yeasts and molds. Yeasts and mold counts on butter and on cottage cheese were generally slightly higher with potato dextrose agar plus aureomycin than with acidified potato detrose agar. Aureomycin causes no precipitation of casein in milk, cream, or cottage cheese, and the plates poured from low dilutions of these materials are free from the precipitate commonly encountered when acidified agar is used.

M43. A semi-quantitative spectrographic analysis of the trace elements in whole milk. C. W. GEHRKE, J. M. BAKER, H. E. AFFSPRUNG, and E. E. PICKETT, Univ. of Missouri, Columbia.

A semi-quantitative spectrographic analysis was made on fall and spring samples of whole milk from Brown Swiss, Holstein, Jersey, and Guernsey cows. Each sample contained fore milk, middle milk, and final milk. All cows were Bang's and mastitis-free. All glassware was thoroughly washed with an alkaline detergent and rinsed in distilled water, double distilled hydrochlorie acid, and double distilled water. Extreme caution was used to prevent contamination. The milking was done directly into large Erlenmeyer flasks. The samples were ashed at 450° C. in vitreosil dishes. A Jarrell-Ash grating spectrograph with a Wadsworth mounting was used. The operating characteristics were d.c. arc., 50 v., and 5 amp. during exposure.

The elements found and their concentration range in p.p.m. in the milk ash of the 4 breeds of cows are as follows: Sr, 1-10; Zn, 30-300; Mo, 10-20; Cu, 50; Al, < 1; Fe, 20-50; Mn, 5-50; and Ag, 2-5. Boron was present in all samples. The following elements were absent in all samples: Pb, Ge, Sn, Bi, Zr, V, Cr, Ni, Ba, Co, Tl, Li, Ti, Cd, and Be. Dithizone and

8-hydroxy quinoline concentration schemes also were employed.

M44. The xanthine oxidase (Schardinger's enzyme) content of dairy products and its inhibition by heat. G. R. GREENBANK, Washington Utilization Research Branch, USDA.

It has been estimated that each liter of milk contains 160 mg. of xanthine oxidase enzyme. The concentration is usually determined by the reduction of methylene blue with hypoxanthine as a substrate. The concentration in milk, cream, skimmilk, whey, centrifuged casein and serum has been determined. Other determinations are in progress. It has been found that the enzyme is more resistant to heat in cream than in milk. Heating milk to 160° F. for 50 min. does not entirely inhibit methylene blue reduction. Heating milk to 170° F. for 15 min. or 225° F. for 15 sec. does inhibit reduction, but heating 40% cream to 170° F. for 35 min. does not destroy all activity.

M45. The effect of xanthine oxidase on the properties of dairy products (a preliminary report). G. R. GREENBANK, L. V. ROGERS, C. F. HUFNAGEL, and M. B. MIDDLETON, Washington Utilization Research Branch, USDA.

Xanthine oxidase is not specific for the oxidation of xanthine or hypoxanthine, but it promotes the oxidation of numerous aldehydes. Through a combined action of this and other enzymes in milk it is possible to postulate a series of reactions to explain the formation of hydrogen peroxide as an end product. It is possible therefore that this enzyme could be of great significance in the problems of deterioration of milk and milk products. The probability of the importance in this respect is supported by the fact that the thermal inhibition of this enzyme is near the conditions and time used in the processing of dried milk for good keeping quality as well as good baking quality. It is also near the temperature and time of heating required to produce good frozen cream. The addition of inhibitors for xanthine oxidase to pasteurized milk before drying resulted in a dried milk of superior keeping quality to that prepared from pasteurized milk without the inhibitor present and almost equal to that prepared from milk heated to 185° F. for 15 min. Furthermore, it has been found that the binding action of raw milk in bread is greatly reduced when inhibitors are added. The results obtained make it appear that the inhibition of this enzyme by heat treatment of the milk used is a factor in producing a good loaf of bread.

M46. Characterization of milk amylases. E. J. GUY and ROBERT JENNESS, Univ. of Minnesota, St. Paul.

The amylases of milk were previously shown to reside in the "lactoglobulin" fraction of the milk serum proteins and to consist of α (liquefying) and β (saccharifying) enzymes. These enzymes have been concentrated and characterized with respect to the effects of temperature, pH, and calcium concentration on their activity.

The amylases apparently are not involved in the depression of loaf volume noted when unheated milk serum proteins or "lactoglobulin" are incorporated into the bread. This conclusion is based on the facts that *(a)* "lactoglobulin" fractions high in amylases did not depress loaf volume, *(b)* commercial α- and β-amylases baked at the level of amylase in "lactoglobulin" did not depress loaf volume, and *(c)* both α- and β-amylase activity in skimmilk and "lactoglobulin" are inactivated by lower heat treatments than required to improve loaf volume.

M47. The effect of heat on the chemical nature of the material adsorbed on the milk fat globule.[1] MORRISON LOEWENSTEIN[2] and I. A. GOULD, The Ohio State Univ., Columbus.

Whole milk and the skimmilk portion of reconstituted milk were given heat treatments of *(a)* momentary warming to 40° C., *(b)* heating to 62° C. for 30 min., or *(c)* heating to 82° C. for 15 min., after which the material adsorbed on the surface of the fat globules was recovered by the cream washing procedure.

The average protein contents of the adsorbed materials recovered from samples of whole milk receiving the specified heat treatments were *(a)* 21.86%, *(b)* 15.54%, *(c)* 7.70%; the same samples had lipid contents of *(a)* 70.65%, *(b)* 77.38%, *(c)* 90.46%. Ash, calcium, phosphorus, and sulfur contents of the samples were in proportion to their protein content. For these samples the phospholipid content of the lipid portion of the adsorbed material was *(a)* 12.10%, *(b)* 8.95%, *(c)* 3.64%.

Heating only the skimmilk portion of the milk resulted in changes in the composition of the adsorbed material similar to those found when the whole milk was heated; however, the changes were even more pronounced.

[1] Funds for this research were provided by the Ohio Dairy Products Research fund and the Oklahoma Agricultural Experiment Station.

[2] Present address: Stillwater, Okla.

M48. The lipolytic enzymes of raw skimmilk.[1] D. P. SCHWARTZ, I. A. GOULD, and W. J. HARPER, The Ohio State Univ., Columbus.

Some basic characteristics of the lipolytic enzyme system of milk have been studied by using freeze-dried skimmilk as the lipase source and milkfat as the substrate. The pH was controlled with an automatic titrimeter, and the lipase activity was measured by a rapid chromatographic technique after incubation of the milk for 0.5 to 6 hr. at 32° C.

The lipase activity was characterized by 3

[1] Supported in part from funds granted to the Ohio State Univ. by the Research Foundation for aid in fundamental research and by the Ohio Dairy Products Research Fund.

independent methods; the influence of pH, the effect of variation in the substrate concentration, and the effect of varying concentrations of formalin, which was found to be a competitive inhibitor. The results of each of these 3 studies substantiated the presence of three different lipases, with pH optima at 7.0, 7.5 to 7.9, and 8.5.

The time of incubation and the substrate concentration were found to be important in evaluating the lipase activity data, since these 2 factors materially influenced the activity at different pH levels.

The end products inhibited the lipase activity at all pH levels. The percentage of fat hydrolyzed decreased as the substrate concentration was increased.

M49. Homogenization and deaeration of milk by ultrasonic waves. MARIE BURGER and W. C. WINDER, Univ. of Wisconsin, Madison.

The content of oxygen, nitrogen, and carbon dioxide in milk was reduced by ultrasonic treatment. The amount of ultrasonic energy needed to accomplish this was small. The higher the frequency of the ultrasonic waves, the more efficient was the removal of these gases.

Cavitation appeared to be necessary for the homogenization of milk. Homogenization of the milk was obtained wth low acoustic intensities. These findings are in agreement with the deaeration studies. The content of gases in the milk controlled the formation of cavitation. Prolonged treatment resulted in agglomeration of fat globules.

Ultrasonic treatment of milk at low intensity caused the small fat globules to be oriented into lines. Under the microscope the fat globules thus oriented appeared to be trapped in the serum, as indicated by their relatively fixed position. These fat globules may be enmeshed within a minor protein coagulum of casein.

Ultrasonic treatment prevented the development of oxidized flavor in pasteurized milk. This may be attributed to the removal of oxygen through the degassing process. However, all the treatments resulted in a hot-oil, rubbery flavor.

M50. Decarboxylation products of amino acids in Cheddar cheese. G. J. SILVERMAN and F. V. KOSIKOWSKY, Cornell Univ., Ithaca, N. Y.

Analyses of 15 commercial Cheddar cheeses showed that a number contained variable amounts of γ-amino-butyric acid and another compound tentatively identified as the amine, cadervine. In other Cheddar cheeses of approximately the same degree of ripening these compounds were not observed.

In a series of 7 experimental cheeses, ripened over a 6-mo. period at 60° F., 2 lots made from raw milk were characterized by the development of an intensive unclean flavor, H_2S odor, and gas holes. The other 5 lots of cheese all made from the same milk but pasteurized were of good quality. Two dimensional paper chromatographic analyses and the Crumpler basic copper carbonate technique for amines and for β- and γ-amino acids indicated the presence of large amounts of γ-amino-butyric acid and the compound tentatively identified as cadervine in the poor quality raw milk cheese, whereas only traces of the former compound and none of the latter were observed in the good quality pasteurized milk cheese.

Of the 6 amino acids capable of being decarboxylated by specific bacterial enzymes, evidence is available that under certain circumstances decarboxylation products from at least 3 amino acids, tyrosine, glutamic acid, and lysine, are present in ripening Cheddar cheese. These products would be tyramine, γ-aminobutyric acid, and cadervine, respectively, along with accompanying carbon dioxide. As the presence of natural milk decarboxylases has not yet been clearly demonstrated, it is suggested that bacterial decarboxylases in cheese are mainly responsible for the production of these products.

M51. Application of ion exchange chromatography to the measurement of free amino compounds in various lactic acid starter cultures.[1] M. K. HAMDY, W. J. HARPER, H. H. WEISER, and G. L. STAHLY, The Ohio State Univ., Columbus.

Moore and Stein's polystyrene ion exchange resin method *(J. Biol. Chem.*, **192**, 1951) was modified to adapt the technique of chromatographic fractionation of amino compounds to routine analysis. The following modifications were made: *(a)* increasing the length of the Dowex 50 column from 100 to 110 cm.; *(b)* changing the pH of sodium citrate buffer from 3.42 to 4.0; and *(c)* eluting at room temperature with a flow rate 1 ml. fraction per 3-4 min. This procedure permitted quantitative analyses in 12-15 hr. of the following 10 acidic amino compounds, listed in order of their elution from the column: cysteic acid, taurine, aspartic acid, threonine, serine, asparagine, glutamine, glutamic acid, proline, and glycine.

The method was applied to determine these compounds qualitatively and quantitatively in the cultures of several lactic acid starter organisms *(Lactobacillus bulgaricus, L. lactic* and *Streptococcus thermophilus.)* The cultures were inoculated singly and in combination into sterile skimmilk, incubated at 42° C., and stored at 10° C. Analyses at various times revealed: *(a)* that there was wide variation in the concentration and type of free amino compounds present in different cultures on prolonged storage, *(b)* there was pronounced effect of one culture on the other, and *(c)* certain compounds were present in all cultures.

[1] Supported in part by funds from the Institute of Nutrition and Food Technology, The Ohio State Univ., The Research and Marketing Act of 1946, and The Ohio Dairy Products Fund.

M52. Fatty acid chain-length specificity of some microbial lipases. J. C. WILCOX, W. O. NELSON, and W. A. WOOD, Univ. of Illinois, Urbana.

The lipolysis of butterfat by *Geotrichum candidum* heretofore has been considered to be nonspecific in relation to the chain-length of the fatty acids liberated in the hydrolytic process. The absence of butyric rancidity in butterfat substrates supporting growth of *G. candidum* has been attributed to the ability of the organism to metabolize the volatile fatty acids as soon as they are liberated. Previous indirect evidence has supported such an hypothesis.

This problem has been reexamined, using cell-free culture filtrates as a source of lipase in order to eliminate the confounding influence of growth. Gross lipolysis was measured by titrating the fatty acids liberated from buffered butterfat substrate by the action of lipase preparations under standard conditions. The volatile acids were separated from the reaction mixture by the McAnally steam distillation procedure. Distillates were concentrated and analyzed for free fatty acids by the Reid and Lederer method of paper partition chromatography.

Although appreciable lipolysis occurred in butterfat reaction mixtures containing *G. candidum* lipase, volatile acids were not detected when distillates were titrated and chromatographed. In contrast, *Candida lipolytica* reaction mixtures contained considerable volatile acidity, and butyric and caproic acids were detected readily by paper chromatograms. These results indicate that microbial lipases possess R-group specificity for the fatty acids occurring in natural mixed triglycerides.

M53. Evaluation of the acid degree of fat and extraction-titration methods for the estimation of free fatty acids in rancid milk and cream. N. P. TARASSUK and E. N. FRANKEL, Univ. of California, Davis.

A study of the partition of free fatty acids among different phases during the churning of rancid cream revealed that in addition to the loss of the water-soluble fatty acids in the serum phase, as much as 30% of the water-insoluble fatty acids was lost. Experiments with model systems of cream emulsions made with fat to which known fatty acids were added established the presence of water-insoluble free fatty acids in the foam phase. The concentration of these acids in the foam can be explained by their high surface activity. Additional losses may occur through mutual solubility of water-soluble and water-insoluble fatty acids.

These data emphasize the limitations of the acid degree of churned fat and the advantage of the direct extraction-titration methods for the estimation of free fatty acids. Different extraction-titration methods were studied with respect to the optimum amounts and ratio of solvents and the pH necessary to secure the greatest recovery of free fatty acids.

M54. Reichert-Meissl numbers of butterfat produced in Kansas. W. D. RUTZ, W. H. MARTIN, and C. H. WHITNAH, Kansas State College, Manhattan.

During the month of Aug. 171 samples of ice cream were collected, which were representative of practically all of the ice cream manufactured in the state and were assumed to contain representative late summer butterfat. Butter oil was prepared from the ice cream samples by a de-emulsification and churning procedure with Minnesota Babcock reagent as the de-emulsifying agent. Reichert-Meissl numbers were determined according to the official methods of the A. O. A. C.

Of 171 samples 2 had Reichert-Meissl numbers of less than 9, indicating gross adulteration. The Reichert-Meissl numbers of butterfat from the remaining samples ranged from 27.40 to 31.35 with an arithmetical mean of 29.19. The Reichert-Meissl numbers of 160 to 169 samples (94.67%) were between 28.00 and 30.50.

Samples of butter were also collected from 10 butter producing plants in Kansas each month for a period of 1 yr., beginning April 15, 1953. The data showing seasonal variation of Reichert-Meissl numbers were completed March 15, 1954.

M55. Lactones and the coconut-like off-flavor of milk fat. P. G. KEENEY, C. T. HERALD, and S. PATTON, The Pennsylvania State Univ., State College.

Evidence from this laboratory has indicated that lactones, principally δ-decalactone, are responsible for coconut-like off-flavors which develop during storage deterioration of certain dairy products. Anhydrous milk fat and dry whole milk are particularly susceptible to the defect. The monounsaturated C_{10}, C_{12}, and C_{14} fatty acids are a logical source of the lactones. Lactone formation appears to be best explained by shifting of double bonds in the acids followed by ring closure. The mechanism of this reaction appears to be nonoxidative. Vacuum packing of dry whole milk does not hinder the development of the coconut-like off-flavor. Hydrogenation of milk fat or low temperature storage of products will effectively prevent the defect, whereas high temperature processing and storage at elevated temperatures aggravate it.

M56. Detection of milk fat adulteration. MARK KEENEY, Univ. of Maryland, College Park.

The use of the chromatographic determination of butyric acid for this purpose will be discussed. Seasonal and geographic data on the butyric acid content of milk fat will be presented.

A modification of the Critical Temperature of Dissolution, which may be used as a rapid screening test, to pick out suspicious samples, will be presented.

M57. Reliability of the Reichert-Meissl and Polenske methods for detecting substitute fats in ice cream. G. M. KLOSER and J. J. SHEURING, Univ. of Georgia, Athens.

The purpose of this study was to determine the accuracy of detecting small amounts of substitute fats in ice cream mixes by using the Reichert-Meissl and Polenske methods.

A popular brand of oleo and 2 commercial vegetable fats, which are on the market for use in making frozen desserts, were used. Butter was churned from cream from the University herd milk, and to this was added 1, 2, 3, 5, and 10% of the substitute fats. Over 400 samples were analyzed, and the amount that could be detected was determined to be 4 to 5% of the total amount of fat.

Ice cream mixes were made with 5, 10, 20, and 30% of the fat content consisting of the substitute fats. The fat was extracted by ether extract method, and the Reichert-Meissl and Polenske values were determined.

M58. Compounds in oxidized milk fat producing the kreis and thiobarbituric acid reactions. MARK KEENEY, Univ. of Maryland, College Park.

Evidence will be presented that these 2 colorimetric tests constitute a class reaction of compounds containing a methylene interrupted dicarbonyl structure. Beta diketones produce yellow chromogens, whereas beta keto aldehydes produce red chromogens.

M59. Effect of heat treatments on antibiotic content of milk.[1] K. M. SHAHANI, I. A. GOULD, H. H. WEISER, and W. L. SLATTER, The Ohio State Univ., Columbus.

Studies were undertaken to determine the effects of various heat treatments and aging on penicillin content of milk. Penicillin was determined quantitatively by standardized Disk Assay Method. Quantity of agar poured in each plate and size of inoculum (test organisms) seem to affect the results of the assay method.

Raw milk inoculated with known quantities of freshly prepared penicillin solution was divided into two lots. One lot was maintained raw and the other was heat-treated. Penicillin was determined immediately in both the lots. Samples were further stored at 0-2° C. and assayed for penicillin every 24 hr. for 7 d.

When milk containing 0.15 to 1.26 u. penicillin per ml. was pasteurized at 143° F. for 30 min., 0.0 to 15.0% penicillin was inactivated. Four to 16.0% inactivation (average 10.1%) occurred when penicillin milk was heated at 160° F. for 30 min. Upon storage for 7 d. at 0-2° C., a further loss of 16.7% of activity resulted. When milk containing 0.25 to 1.0 u. penicillin per ml. was autoclaved at 15 p.s.i. for 15 min., 45.0 to 69.0% penicillin was inactivated, with an average of 59.7%. Upon aging for 7 d., a further reduction of 9.9% penicillin occurred.

Ephenamine penicillin G was found to be more heat-labile than procaine penicillin G and potassium penicillin O.

[1] Supported by a grant from the U. S. Public Health Service (National Institute of Health) and by funds from the Ohio Dairy Products Research Fund.

M60. The flavor of fresh concentrated milk. E. S. GUTHRIE, Cornell Univ., Ithaca, N. Y.

Fresh concentrated milk samples were obtained from 6 dairy plants in the United States and Canada. The holding qualities of the 16 samples of the reconstituted concentrated milk remained good to excellent in flavor from 6 to 22 d. at 36 to 40° F.

In the manufacture of fresh concentrated milk the body flavor of the cow, the barny flavor, and the feed flavors, if present, are removed in the vacuum pan. In addition, this study shows that oxidized flavors rarely develop in this product.

M61. The use of a Venturi atomizer in spray drier design. V. H. TOWNLEY and S. T. COULTER, Univ. of Minnesota, St. Paul.

A spray drier has been developed with a venturi atomizer, which atomizes the condensed milk and, at the same instant, mixes the resulting spray with the drying air. Inlet air at temperatures up to 535° F. has been used to produce dry whole milk having a solubility index of less than 0.1 ml. and containing no brown specks or other indications of heat damage.

A venturi with a throat 2 in. in diameter and a diffuser 11 in. long was used. The velocity of the hot air reached about 900 ft. per sec. at the throat of the venturi and then gradually decreased to approximately 200 ft. per sec. at the end of the diffuser. Since the power lost by a sudden reduction in velocity is proportional to the change in velocity squared, $(V_1-V_2)^2$, an efficient diffuser is an important factor in determining the power requirements of a drier using the drying air to atomize the liquid. Without a diffuser, an air pressure of approximately 120 in. of water would be necessary to produce a velocity of 900 ft. per sec., as compared to a pressure of 14 in. of water actually required for the venturi used on this drier.

The use of high temperature drying air increases both the power and heat efficiency of the drying operation and increases the capacity of the drier. Increasing the inlet air temperature from 300° to 500° F. with a constant exit air temperature of 170° F. resulted in an increase of 35% in heat efficiency and more than doubled the powder production.

The power requirements of this drier were less per lb. of water evaporated than that of most commercial driers, since the increased amount of evaporation per cu. ft. of air and the elimination of the high pressure pump more than compensated for the greater fan pressures required.

M62. Oxygen uptake by milk and milk constituents. A. THOMAS, S. T. COULTER, and R. JENNESS, Univ. of Minnesota, St. Paul.

The O_2 uptake of milk and other systems at 165° F. for periods up to 5 hr. was measured with a Warburg respirometer. Whole milk, skimmilk, and cream absorbed approximately the same amount of O_2 per unit volume. Cream samples exhibited greater variation than whole milk and skimmilk. The amount of O_2 taken up by skimmilk was significantly greater than the total amount required to oxidize both the protein –SH to –SS– and the ascorbic acid to dehydroascorbic acid.

Simple systems of fat and casein took up negligible amounts of O_2. Lactose solutions (of 1 lot of lactose) exhibited large uptakes of O_2 which were suppressed by ion exchange or addition of disodium dihydrogen Versenate or sodium caseinate. Milk serum protein preparations varied in their ability to take up O_2, as did crystalline β-lactoglobulin. These variations apparently are due to differences in metallic ion concentrations.

M63. Use of sucrose in the preservation of frozen condensed skimmilk. T. J. MUCHA and R. W. BELL, Washington Utilization Research Branch, USDA.

Sucrose was added to skimmilk, before and after concentrating the m.s.n.f. to 3:1, 4:1 and 5:1 ratios at the rate of 5, 10, and 20%, based on the weight of the concentrate, and the products were stored at –17°, –22°, and –27° C.

When sucrose was added before condensing, the viscosity of the thawed concentrate was increased relative to the comparable sugar-free samples; the reverse was true when sucrose was added after concentrating. As the s.n.f. concentration was increased, more sucrose was required in order to obtain a proportionate decrease in viscosity. Except for the added sweetness, there were only slight differences in the flavor of these samples from that of the controls.

The addition of 5% sucrose to 3:1 and 4:1 condensed skimmilk and of 10% to the 5:1 product yielded milks which were more fluid after storage at –17° C. than comparable samples having no added sugar that were stored at either –22° or –17° C. There was no advantage in adding sucrose when the storage temperature was –27° C.

M64. A mechanical low energy method for determining ease of dispersion of milk powder. W. K. STONE, F. S. HIRT, K. R. WOOD, and J. M. MCINTIRE, Food and Container Institute for the Armed Forces, Chicago.

This method utilizes low energy mechanical stirring and permits measurement of ease of dispersion of both nonfat and whole milk powder. The stirring unit consists of an adapted model *B* Hobart Kitchen Aid equipped with an automatic switch and is operated at 54 r.p.m. A tube was fitted into the bottom of the mixing bowl to permit draining of the milk. The unit was mounted on a stand 12 in. high.

Fifty-two g. of powder are added to 400 ml. of water and the automatic switch is started agitating at the instant the mixers contact the water. After a specified stirring time, the outlet tube in the bowl is opened and the milk is allowed to flow through a 210-μ screen. Total solid determinations are run on an aliquot, and the total g. dispersed is the dispersion value for this stirring time.

The amounts of powder dispersed during 10, 20, 30, and 40 sec. stirring represent ease of dispersion for a powder. Precision was determined for each of the above stirring times, and a formula was developed for calculating values for any other stirring time from a measured value obtained for any single stirring interval. Data on the influence of temperature, storage, and surface active agents on this dispersing property will be presented.

M65. Dispersibility of spray-dried milk powder as affected by manufacturing conditions. U. S. ASHWORTH, J. M. MCINTIRE, GLEN WATSON, HANS GUNTHARDT, and H. A. BENDIXEN, State College of Washington, Pullman.

The optimum manufacturing conditions for maintaining a high dispersibility (wettability) for dried milk during storage at 85° F. for 4 mo. were found to be as follows:

1. A fat content of 5-10% in the powder, although very efficient homogenization of condensed milk (3:1) allowed the level of fat to be raised to 26%.
2. The efficiency of homogenization as measured by turbidity (*J. Dairy Sci.*, **34**: 317) was closely correlated with wettability in the range between a K value of 1.9 and 2.6. In the fat range above 26% there was a loss of wettability with each increment of fat regardless of the efficiency of homogenization, although within each fat range the wettability increased with the value of K.
3. The optimum condensing ratio was about 3.5 to 1 but varied some with the fat content of the milk. In the fat range between 12 and 20% (in the powders) the condensing ratio was of more importance than the homogenization efficiency as a means for improving the wettability.
4. Within certain limits there was no apparent effect on wettability of either the preheat treatment of the original milk or the temperature of the drying air.
5. A detrimental effect on wettability was observed whenever the moisture content was below 1.5% or above 4.5, the optimum moisture level being about 3.5%.

6. By passing the homogenized condensed milk through a centrifugal separator the homogenization efficiency and wettability could be improved, providing some separation of the fat was achieved.

M66. Comparison of baking quality of frozen condensed and dried skimmilk. L. V. ROGERS and T. J. MUCHA, Washington Utilization Research Branch, USDA.

The baking quality of nonfat milk solids stored in the dried and in the frozen condensed state after 2 conditions of heat treatment of the skimmilk was compared over a period of 1 yr. Throughout this period the high-heat-treated (80° C. for 30 min.) skimmilk solids preserved in a frozen or dried state produced larger loaf volumes and better bread than the low-heat (62.2° C. for 30 min.) solids. The advantages in loaf volume with skimmilk solids stored in a dried state over those stored in a frozen state, although small, indicate there are factors in frozen storage of condensed skimmilk which impair baking quality. However, the heat treatment of the skimmilk was the primary factor in determining baking quality.

PRODUCTION SECTION

P1. Fertilization and embryonic mortality rates for bulls with histories of either low or high fertility in artificial breeding. H. J. BEARDEN, WM. HANSEL, and R. W. BRATTON, Cornell Univ., Ithaca, N. Y.

In order to compare the fertilization rates and embryonic mortality rates of bulls with histories of either low or high fertility in artificial breeding, virgin heifers are being bred artificially to bulls so classified. Half of the heifers are slaughtered at 3 d. and half at 33 d. after breeding. Seventy-two heifers have been bred and slaughtered to date. In those slaughtered at 3 d., normal cleaved ova have been recovered from 80.0% of 15 heifers bred to bulls of low fertility, and 95.5% of 21 heifers bred to bulls of high fertility. In heifers slaughtered at 33 d., normal embryos have been recovered from 46.7% of 15 heifers bred to low fertility bulls, and 80.9% of 21 heifers bred to high fertility bulls. Thus, the embryonic mortality rates are 33.3% and 14.6%, respectively, for heifers bred to bulls of low and high fertility. Although the difference between these embryonic mortality rates (18.7%) is rather large, it is not statistically significant with the number of animals involved to date. The 28-35 d. nonreturns to 1st services in artificial breeding for the ejaculates used to breed these heifers were 68.0% for the low fertility bulls and 83.4% for the high fertility bulls.

P2. Field trials with semen containing various combinations of antibacterial agents. E. L. WILLETT and J. OHMS, American Fdn. for the Study of Genetics, Madison, Wis.

A field trial with over 104,000 services and 90 bulls in 5 studs widely separated geographically was carried out *(a)* to compare fertility of semen without antibacterial agents with fertility when 3 different combinations of antibacterial agents were added to yolk-citrate diluent and *(b)* to determine how many bulls were benefited by antibacterial agents. The collections were not split, and each treatment was replicated 3 times with each bull. Thus, each bull was represented in the experiment by 12 collections. The 28-35 d. nonreturns from first services performed on the day after collection were as follows: No antibacterial agents, 78.1%; sulfanilamide (0.3 g/100 ml) and dihydrostreptomycin (500 units/ml), 81.4%; streptomycin, 79.6%; and potassium penicillin G (500 units/ml) and streptomycin, 79.0%. These differences were not significant. Corresponding figures for 60-90 d. nonreturns were 63.2, 70.2, 67.0, and 67.2%, respectively. Here the only differences not significant were those between sulfa plus streptomycin and streptomycin and between streptomycin and penicillin plus streptomycin. The 60-90 d. nonreturn rates for 23 bulls differed significantly between the no-drug and the sulfa-streptomycin treatments as contrasted with an expectancy of 4.5 bulls at the 5% level of probability.

P3. Some of the influences of mixed ejaculates upon bovine fertility. E. A. HESS, TOM LUDWICK, H. C. RICKARD, and FORDYCE ELY, The Ohio State Univ. and Ohio Agr. Expt. Sta., Columbus.

Some evidence indicates that properties in male accessory fluids contribute to the fertilizing capacities of an ejaculate. A study was initiated to determine reasons for the difference in various ejaculates from the same bull or different bulls due to variations in secondary sexual secretions.

The first part of the experiment involved a laboratory appraisal of 51 mixed semen samples (3 to 4 ejaculates per mixture) from 47 bulls. The technique consisted of mixing samples from 3 to 4 bulls' ejaculates in equal proportions. Microscopic evaluations of motility and per cent alive were made at 6 hr., 2 d., 4 d., and 7 d. The semen mixing resulted in greater motility and per cent alive as compared to the regular unmixed semen samples. Statistical analysis indicated significance at the 1% level.

The second phase of this study included a field trial in which nonreturn data for mixed semen were compared to nonreturn data of regular semen. Conception rates were based on 30-60 d. nonreturns, and the data were summarized from 364 first and 65 repeat services for mixed semen; 1,494 first and 701 repeat services for regular semen. The difference between the mixed semen nonreturns and control nonreturns was 15.7% for Holsteins and 12.3% Guernseys. This increase in conception with mixed ejaculates may be somewhat magnified because of the small number of cows bred, but significant trends are definitely indicated.

Results from mixed semen data may be used in semen physiology research. This includes the role of accessory glandular secretion variations in different bulls and different ejaculates. This technique may have direct practical applications to semen diluent preparations.

P4. The use of frozen skimmilk as a semen diluter. VICTOR HURST and J. P. LAMASTER, South Carolina Agr. Expt. Sta., Clemson.

An experiment was designed to compare 3 semen diluters: frozen egg yolk–sodium citrate (FEYC); frozen skimmilk (FSM); and freshly prepared skimmilk (SM). The frozen diluters had 500 u. of penicillin and 500 γ of streptomycin added per ml. of diluter prior to freezing. The freshly prepared skimmilk contained the same concentration of antibiotics. Frozen diluters were stored at –30° C. for 1 mo. prior to use. Skimmilk diluters were prepared by heating fresh pasteurized skimmilk to 92-95° C. for 1 min. The egg yolk–citrate diluter was made up of 20% egg yolk-80% 0.1 *M* sodium citrate. All semen was diluted 100 times. Cows in the Clemson Dairy Dept. herd served as experimental animals. Results were based on pregnancy examinations made 3 mo. after insemination. The following conception rates were obtained: FEYC, 8 inseminations resulting in 5 pregnancies; FSM, 10 inseminations resulting in 7 pregnancies; SM, 10 inseminations resulting in 3 pregnancies. The results indicate that frozen skimmilk may be successfully used as a bovine semen diluter.

P5. The longevity of bovine spermatozoa in chemical and heat-treated pasteurized milk. E. N. BOYD, J. R. PERKINS, DURWARD OLDS, and D. M. SEATH, Kentucky Agr. Expt. Sta., Lexington.

A study was made of the effect of sulphydryl compounds in milk upon the livability of bovine semen. More than 200 samples of semen were diluted with milk which had received various treatments and had been stored at 5° C. Motility was observed daily under the microscope for as long as motility existed.

The livability of the semen was determined in pasteurized milk, and in pasteurized milk which had been heated to 80, 85, 90, and 95° C., both momentarily and for 10-min. holding periods. Also, the livability of semen was determined in pasteurized milk which had sulphydryl compounds added by *(a)* saturating the pasteurized milk with H_2S by aspiration; *(b)* addition of cysteine (free base) at several concentrations; and *(c)* addition of thioglycolic acid at several concentrations.

In this study it was confirmed that bovine spermatozoa will not survive for more than 1 or 2 d. in pasteurized milk, as such. When pasteurized milk was heated momentarily to various temperatures, the lowest temperature of those studied at which the semen survived for a desirable length of time was 90° C. For samples held for 10 min. at various temperatures, no difference in livability of the sperm was observed. Thioglycolic acid, a sulphydryl-containing compound, when added to milk, gave results superior to those of pasteurized milk heated to 95° C. for 10 min. Beneficial results were obtained when other compounds were added, but to a lesser degree.

P6. The effect of yolk and citrate levels and stage of maturity on the survival of bull sperm at sub-zero storage temperatures. W. C. KINNEY, JR., and N. L. VANDEMARK, Univ. of Illinois, Urbana.

In attempts to determine the optimum level of yolk for freezing bull sperm at –79° C., the percentage of yolk was varied at the expense of the citrate solution in a yolk-citrate 7% glycerol medium. Optimum sperm "freezability" was obtained when 16 to 32% yolk was used. When the percentage of yolk was varied and the citrate salt concentration held constant, freezability was highest with 16 and 24% and significantly lower with 32% yolk. The optimal percentage of citrate in the final mixture was between 1.55 and 1.95% (wt. per vol. of Na-citrate dihydrate). This mixture was obtained by diluting the semen first with a 1:1 yolk to citrate extender (2.9% cit.) then glycerolating with an equal volume of a 14% glycerol solution in 2.9% citrate buffer.

The stage of maturity of sperm appears to affect their freezability. With first and second ejaculates, collected a few minutes apart, the percentage of sperm surviving freezing was highest in the second ejaculates. When the freezability of 20 consecutive ejaculates (collected in a 4-hr. period) was compared, sperm survival tended to increase up to the 4th ejaculate and decreased thereafter. Epididymal sperm exhibited the poorest resistance to the freezing process.

P7. The effect of cysteine hydrochloride on the livability of bull spermatozoa in unheated skimmilk. P. E. JOHNSON, R. J. FLIPSE, and J. O. ALMQUIST, The Pennsylvania State Univ., State College.

Studies have shown that raw or pasteurized skimmilk is spermicidal when used as a diluter for bull semen, whereas milk heated at 92-97° C. for 10 min. is equal to egg yolk–citrate diluter in supporting livability and fertility. In further studies of the use of milk as a diluter, the addition of cysteine hydrochloride to pasteurized skimmilk has been found to produce livability results equivalent to those obtained by heating pasteurized skimmilk at 92° C. for 10 min. Eight concentrations of cysteine hydrochloride ranging from 0 to 4 mg. per ml. were tested. Optimum livability was obtained at the 1 mg. per ml. level.

Twenty-three ejaculates of high quality semen

were diluted to a final concentration of 15×10^6 motile spermatozoa per ml. in unheated skimmilk containing cysteine hydrochloride at a level of 1 mg. per ml. Diluted samples were stored at 4° C. and motility estimations made after 1, 2, 3, 4, 6, 8, 10, and 12 d. of storage. No apparent difference in spermatozoan livability was found between semen samples treated with cysteine and control samples heated at 92° C. for 10 min. Preliminary trials indicate that glutathione acts in a similar manner.

It is postulated that cysteine hydrochloride, and other sulfhydryl compounds, may act by lowering the oxidation-reduction potential of the milk, thus inactivating lactenin, an antistreptococcal and apparently spermicidal substance found in milk.

P8. Activation of bovine spermatozoa by the use of sodium carbonate. H. C. RICKARD, TOM LUDWICK, E. A. HESS, and FORDYCE ELY, The Ohio State Univ., Columbus.

In this study an attempt was made to develop a technique for activating spermatozoa prior to insemination in order to increase conception rates. Sixteen semen samples were adjusted to various pH concentrations (6.5, 6.8, 7.1, 7.4) with sodium carbonate and citric acid and held at specific temperatures. Observations were made at various intervals to determine the optimum pH for maximum motility and livability. It was found that a weak concentration of sodium carbonate would greatly increase motility for an extended period of time at certain temperatures.

A field trial was conducted with 8 technicians from C.O.B.A. stud. The technicians bred alternate first services with activated and normally diluted semen. Over a period of 2 mo., 666 first services were completed with activated semen. For the control group 1,143 services were made.

Analysis of the nonreturn data for 1-day-old semen showed an insignificant difference between the two groups. However, the 2-day-old nonreturns showed a difference of 10% between the activated group (70.33%) and the control group (60.34%).

It was concluded that considerable increase in conception may be produced by activating spermatozoa with the proper amounts of sodium carbonate.

P9. The effect of antibiotics, levels of glycerol, and rates of freezing on revival rate of bovine spermatozoa in yolk-citrate and milk extenders. W. E. ERICKSON, E. F. GRAHAM, and E. C. FREDERICK, Univ. of Minnesota, St. Paul.

Freezings were conducted by using split sample comparison of semen diluted 1:40 with no antibiotics, 500γ of streptomycin/ml of diluted semen, 500 u. of penicillin/ml of diluted semen, and both antibiotics at stated levels. Glycerol levels of 7 and 10% were used for both yolk-citrate (2.9%) and milk. Rate of cooling for yolk-citrate was 3° C./min from 5° to –15° C.; 3° and 5° C./min to –30° C.; and 5° C./min to –79° C. Rate of cooling for milk was 2° C./min from 5° C. to –15° C.; 2° and 5° C./min to –30° C.; and 5°/min to –79° C.

Sufficient samples were frozen to permit checking immediately after freezing and every 2 wk. for 8 wk. Recovery after freezing indicates no injurious effect due to addition of antibiotics at prescribed levels, with slightly better recovery obtained when both antibiotics were used. Best recovery with yolk-citrate (2.9%) was obtained by using both antibiotics and 7% glycerol and frozen over critical period at 5° C./min. Best recovery with milk was obtained by using both antibiotics and 10% glycerol and frozen over critical period at 2° C./min.

P10. The relationship of diluter composition and glycerol equilibration time to survival of bull spermatozoa after freezing to –75° C. JACK SAROFF and J. P. MIXNER, New Jersey Agr. Expt. Sta., Sussex.

The survival of bull spermatozoa after freezing to –75° C. was determined in a 4×4 factorial design experiment (8 semen samples) with 4 basic semen diluters and 4 levels of glycerol for each diluter. The over-all mean motility percentages for the 4 basic diluters were as follows: 43.75 to 47.5% egg yolk and citrate, 7.5; 17.5 to 19.0% egg yolk and citrate, 44.4; boiled skimmilk, 41.3; and boiled whole homogenized milk, 47.0. The corresponding over-all mean motility percentages for the 5.0, 7.5, 10.0 and 12.5% glycerol levels (by vol.) were 35.5, 39.2, 35.4, and 30.2, respectively. The diluter which gave the highest mean motility (51.8%) had the following final concentration: 18.5% egg yolk, 2.22% sodium citrate dihydrate, and 7.5% glycerol.

A 3×4 factorial design experiment (6 semen samples) was performed with 3 levels of egg yolk (18.4, 23.0, and 27.6%) and 4 glycerol equilibration times (2, 6, 12, and 18 hr.). Glycerol and sodium citrate levels were kept constant at 8 and 2.175%, respectively. The over-all mean motility percentages for the 18.4, 23.0, and 27.6% egg yolk-citrate diluters were 34.9, 34.5, and 30.7%, respectively. The over-all mean motility percentages for the 4 glycerol equilibration times were as follows: 2 hr., 26.7; 6 hr., 32.1; 12 hr., 36.7; and 18 hr., 37.8.

P11. Preliminary fertility results with frozen bovine spermatozoa. R. W. BRATTON, R. H. FOOTE, and JOAN C. CRUTHERS, Cornell Univ., Ithaca, N. Y.

The fertility of bovine semen frozen in a concentrated form (200×10^6 motile sperm per ml. extender), thawed at 5° C., extended to final volume, and used for breeding within 24 to 48 hr. was compared with that of semen frozen in single breeding samples. The latter was

thawed just before being used for insemination. The split sample technique was used with 2 ejaculates from 3 different bulls. Prefreezing processing and freezing procedures were essentially as described by Polge *et al.* and Dunn *et al.*, with certain modifications. Unfrozen semen processed in the conventional manner served for the control breedings. All semen was extended to provide between 8 and 10 million motile spermatozoa per insemination at the time of insemination, though estimates at this time indicated that actual numbers varied between 4 and 14 million. The 28-35 d. % nonreturns to first services were as follows: unfrozen, 459 services, 81.1%; frozen concentrated, 432 services, 66.5%; frozen as single breeding samples and stored 1 d. at –79° C., 55 services, 85.5%; frozen as single breeding samples and stored 103 d. at –79° C., 92 services, 86.8%. Incomplete, 60-90 d. % nonreturns indicate that the fresh unfrozen semen will have the highest average fertility rate.

P12. Effects of levels of sodium citrate, glycerol, and equilibrating time on recovery of bovine spermatozoa after storage at –79° C. R. G. CRAGLE and R. M. MYERS, North Carolina State College, Raleigh.

Optimum levels of per cent sodium citrate and glycerol and the hours of equilibrating time for the survival of bovine spermatozoa after being frozen (–79° C.) for 5 d. were investigated. A composite experimental design with 5 equally spaced levels of each variable, 15 treatments, was employed. Spermatozoa progressively motile (pm) expressed as per cent recovery was the criterion used for evaluating treatments. The 15 treatments carried out on one ejaculate (> 60% pm) from each of 4 Holstein bulls made up one experimental series. After each series, the levels of the 3 variables were adjusted by the method of steepest ascent as indicated by recovery of the spermatozoa. In the first series, the levels used were: for citrate, 2.9 to 4.5%; for glycerol, 5 to 21% (by vol.); and for equilibrating time, 4 to 14 hr. Completion of 5 series indicated acceptable recovery of per cent pm for sodium citrate from 2.4 to 3.3%; for glycerol from 4.5 to 8%; and for equilibrating time from 6.5 to 16 hr. Values as low as 1.6% for citrate, 3% for glycerol, and 4 hr. for equilibrating time and values approximating 4.4% sodium citrate, 21% glycerol, and 28 hr. equilibrating time appear to be detrimental.

P13. Techniques for freezing bull spermatozoa in heated milk and preliminary breeding results. W. T. O'DELL and J. O. ALMQUIST, The Pennsylvania State Univ., State College.

The general procedure consisted of diluting the semen with pasteurized skimmilk (previously heated at 92° C. for 10 min.), cooling to 5° C., gradually adding an equal volume of 20% glycerol in heated skimmilk, equilibrating overnight, and freezing to –79° C.

Five equal additions of glycerol at intervals of either 6 or 12 min. gave revival and survival rates superior to 3 equal additions at intervals of 10 min. or adding the glycerol all at once. No significant differences were found between equilibration periods of 30 min., 4 hr., and 18 hr., and only a significant bull-to-bull variation existed. Glycerol levels of 10 and 13% were slightly better than 7% and significantly better than 4 and 16%. Semen samples thawed in a water bath at 5° and 40° C. gave comparable mean motility revival and survival rates which were significantly higher than for samples thawed at 22° C.

Preliminary fertility results from 15 bulls at Southeastern Pa. Artificial Breeding Coop. were obtained with semen frozen after dilution (24×10^6 motile sperm per ml.) with heated homogenized milk containing 7.5% glycerol plus penicillin and streptomycin. The frozen semen was stored up to 4 mo., thawed at the headquarters, and used for insemination within 3 hr. The 60-90 d. nonreturn rate for 442 cows was 60%. A total of 83 services to one bull with semen frozen following collection by massage yielded a nonreturn rate of 52%.

Thus it appears that either heated homogenized milk or skimmilk can be used as a diluter for freezing bull spermatozoa.

P14. Interference by glycerol with differential staining of bull spermatozoa. J. P. MIXNER and JACK SAROFF, New Jersey Agr. Expt. Sta., Sussex.

The relative usefulness of two measures of spermatozoan viability (per cent of motile spermatozoa and per cent of live spermatozoa as determined by differential staining) was studied on frozen semen with 3 semen diluters containing varying levels of glycerol. One semen diluter was composed of 1 part of egg yolk and 3 parts of a 3% aqueous solution of sodium citrate dihydrate, the other 2 being boiled skimmilk and boiled whole homogenized milk. Glycerol levels, as employed with the 3 diluters, varied from 2.5 to 12.5%. Results indicated that as the levels of glycerol in the diluters exceeded 4%, the per cent of live spermatozoa decreased in a very disproportionate manner to the per cent of motile spermatozoa or the predicted per cent of live spermatozoa, becoming very extreme at the high levels of glycerol. The most plausible explanation of these observations is that the higher levels of glycerol increased the permeability of the living and motile sperm cells to the stain, thus allowing some of them to be counted as dead cells. The per cent of motile spermatozoa is judged to be the better measurement of spermatozoan viability in studies involving the use of glycerol as a constituent of semen diluters.

P15. Experimental rations to study the phosphorus requirement of young dairy bulls for normal growth and semen production. P. E. POITRAS, S. D. MUSGRAVE, M. RONNING, and R. MACVICAR, Oklahoma A. & M. College, Stillwater.

Five bull calves, 4-6 mo. of age, were fed a concentrate (oats 10%, corn 15%, beet pulp 15.5%, blood fibrin 10%, wood pulp 1.5%, Omalass 15%, hominy 29%, bone meal 1%, salt 1%, limestone 1%, and a 1% hominy premix containing a vitamin A-D supplement and aureomycin) and roughage ration (wood pulp 40% and blackstrap molasses 60%, by weight) in amounts calculated to furnish required total protein and TDN. Calcium supplied was in excess of requirements but phosphorus was below N.R.C. recommendations. The concentrate contained 16.3% total protein, 17.94% fiber, 0.886% calcium, and 0.31% phosphorus by analysis. A TDN value of 74.3% was calculated. The roughage contained 3.47% total protein, 30.9% fiber, 0.40% calcium, and 0.054% phosphorus.

A second group of 6 calves, 4 mo. of age, was given a concentrate (oats 9%, corn 15%, beet pulp 20.5%, blood fibrin 12%, wood pulp 1.5%, Omalass 15%, polished rice 24%, salt 1%, limestone 1%, and a 1% rice premix containing a vitamin A-D supplement and aureomycin) and the same roughage as above. This concentrate, as analyzed, contained 16.7% total protein, 6.91% fiber, 0.66% calcium, and 0.098% phosphorus. A TDN value of 72.3% was calculated.

As they grew larger, all calves were given additional beet pulp to increase the fiber content of the ration. In the first group plasma phosphorus and calcium levels, body weight gains, and reproductive development as measured by semen data were satisfactory. In the other group preliminary data indicate a decrease in plasma phosphorus. Digestion trials were conducted with all calves.

P16. Interrelationships among thyroidal activity, sexual behavior, semen production, and the fertility of bulls used in artificial breeding. W. S. GRIFFITH, CECIL BRANTON, T. E. PATRICK, G. F. D'ARENSBOURG, and H. C. KELLGREN, Louisiana State Univ., Baton Rouge.

In view of the lack of objective data on the interrelationships among climatic factors, body temperatures, thyroidal activity, sexual behavior, semen production, and fertility of bulls used in artificial breeding, as well as a lack of a means of objectively rating sexual behavior, a study was initiated in Nov. 1953, which will have a duration of at least 12 mo.

Semen collections are made at weekly intervals from 18 bulls. Two ejaculates are collected from each bull on each collection day. The following measurements of sexual behavior are made at each collection: reaction time; collection interval (the time elapsing between the collection of one ejaculate and the release of the bull for a 2nd collection); excitement score (a 14-point scoring system); thrust score (a scoring system ranging from 1 to 5); and per cent successful services.

In addition to these measurements, semen quality and fertility data are being obtained. Blood samples are taken from each animal at monthly intervals for plasma PBI determinations. Both environmental wet-bulb and dry-bulb temperatures are taken at the time of semen collection. Body temperatures of the bulls will be taken once each wk. from the last wk. in May until the 1st wk. in September.

Data for the first 3 mo. of the experiment will be presented.

P17. Studies on the permeability of the gut of the newborn herbivore. H. F. DEUTSCH and VEARL R. SMITH, Univ. of Wisconsin, Madison.

The permeability of the gut of newborn calves and kids is being studied by measurement of gamma globulins in blood serum with an ultra-centrifuge. Quantitative measurements of the gamma globulins were made at 4-hr. intervals after colostrum was fed to kids and calves within 4 hr. of birth. Usually maximum concentrations appeared 12 hr. after colostrum feeding.

Studies were also made of factors which may affect permeability of the gut. Newborn calves were fed mature milk to 36 hr. of age. Some of the calves were fed mineral oil to prevent constipation. Colostrum was fed at 48 hr. of age. Blood samples were taken at 0, 48, and 60 hr. postnatally. There was no absorption of gamma globulins when colostrum was withheld for 48 hr. A series of calves were then injected either intraperitoneally or intramuscularly with cortisone, ACTH, diethylstilbestrol in oil or an aqueous suspension, progesterone in oil or an aqueous suspension, and a combination of diethylstilbestrol and progesterone at various times before the feeding of colostrum. None of these substances had a measurable effect on the permeability of the gut to gamma globulins.

P18. The value of a combination of antibiotics vs. single antibiotics for young dairy calves. C. A. LASSITER, T. W. DENTON, and J. W. RUST, Kentucky Agr. Expt. Sta., Lexington.

Thirty-two 2-3 day old Jersey and Holstein calves were raised to 84 d. of age on a limited milk-starter-hay system to evaluate a combination of aureomycin and terramycin vs. the single antibiotics for young dairy calves. The aureomycin supplements, Aurofac D and 2A, and terramycin supplement, TM-5, were used. The antibiotic supplements were fed at the rate of 75 mg. of crystalline antibiotic per calf daily in the milk for the first 49 d. and in the starter

at the rate of 24 mg. per lb. of starter thereafter. When the combination of antibiotics was fed, each antibiotic supplied ½ of the total amount fed.

The average daily gains, feed efficiencies, (lb. TDN/lb. gain) and scouring data (total number of days per group) of the calves on the various treatments were as follows: Control — 1.03, 1.96, 6; aureomycin — 1.18, 1.83, 13; terramycin — 1.15, 1.85, 8; aureomycin plus terramycin — 1.13, 1.86, 5. The differences in average daily rate of gain were not statistically significant. The occurrence of scours was not a significant problem and the incidence was probably too small to evaluate properly.

P19. Growth and hematological response of calves to antibiotic and arsenical supplementation. F. G. Owen and R. S. Allen, Iowa Agr. Expt. Sta., Ames.

Five groups of 6 Holstein calves each were fed a whey-product milk replacement, hay, and concentrates from 4 to 88 d. of age. Rations of the various groups were supplemented as follows: I, none; II, terramycin; III, bacitracin; IV, chloromycetin; V, 3-nitro-4-hydroxyphenyl arsonic acid. Rates of supplementation were: antibiotics, 40 mg. daily per calf to 60 d. of age and 80 mg. thereafter; arsonic acid, 50 p.p.m. of total ration. Blood samples were drawn and weights and measurements were taken at weekly intervals.

For each group the mean rates of gain (lb/day) and feed efficiencies (lb. TDN/lb gain), respectively, were: control, 0.78, 2.92; terramycin, 1.04, 2.27; bacitracin, 0.97, 2.36; chloromycetin, 0.86, 2.66; and arsonic acid, 0.99, 2.37. The more rapidly gaining groups attained greater height at the withers, whereas chest and barrel circumference measurements did not differ among groups. There were no appreciable differences among groups in incidence and intensity of diarrhea, which reached maxima during weeks 3 to 5 inclusive, except that both were greatest for the chloromycetin group. No differences among treatments in total erythrocyte, total leucocyte, and differential leucocyte counts were apparent.

P20. The effect of different levels of dietary terramycin on the growth of young dairy calves. G. I. Pritchard, W. H. Riddell, and W. B. Durrell, Univ. of Vermont, Burlington.

Twenty-seven heifer calves were divided into 3 groups and fed whole milk for 6 wk., then skimmilk until 12 wk of age. Good quality hay and a 20% protein concentrate were fed to a maximum of 4 lb. daily. Group I served as the control. Groups II and III received terramycin at the rate of 15 and 60 mg. per 100 lb. of body weight, respectively. The average daily gain for the period was 1.48 lb. for Group I; 1.55 lb. for Group II; and 1.53 lb. for Group III. These differences were not statistically significant. The average gain over the Ragsdale standard was 37.7, 43.5, and 43.1 lb. for the groups, respectively.

The calves were housed in the Experiment Station barn, where an earlier trial had been run with terramycin fed at the rate of 30 mg. per 100 lb. of body weight. In that trial, the controls gained 25.9 lb. and the supplemented calves 40.9 lb. above the Ragsdale standard. The greater gain of the present controls suggests that the calves were not exposed to as many low-grade pathogens as were the earlier calves.

A study of the hemoglobin level and white blood cell differential of 12 calves did not reveal any significant difference between control and supplemented calves.

P21. Effect of aureomycin on certain blood constituents (Hb, packed RBC, Ca, P, RBC and WBC), body temperatures, weights of organs and tissues, and thickness of small intestine. L. L. Rusoff, F. T. Landagora, and H. H. Hester, Louisiana Agr. Expt. Sta., Baton Rouge.

Thirty Holstein and Jersey male calves were divided into 3 groups. Group 1 served as the control, Group 2 received aureomycin orally (50 mg. daily in the milk and a 0.5% level of Aurofac 2A in the calf starter), and Group 3 was administered aureomycin by intramuscular injection (400 mg. weekly). Feeding practices were the same for all groups. Milk was fed for 28 d. and a 20% plant-protein calf starter was fed with alfalfa hay.

Body temperatures were obtained weekly, and blood samples were collected for analyses at 4-wk. intervals. Three or 4 animals from each group were slaughtered at 12 and 16 wk. of age, and organ and tissue weights were obtained. At the time of slaughter both groups of calves receiving aureomycin had gained approximately 17% more weight than the controls.

Preliminary results indicate: a lowered body temperature in Holstein calves receiving aureomycin; no apparent differences between the groups of animals in erythrocyte counts, per cent hemoglobin, per cent hematocrit value, plasma Ca and plasma inorganic P values; and an indication of a decrease in leucocyte counts for the aureomycin supplemented calves. Certain organs (pituitary, thyroid, thymus, liver, etc.) were heavier in the aureomycin calves; however, no differences were observed in organ weights when computed as percentage of body weight. A decrease in thickness of duodenal and jejunal sections of the intestinal wall and an increase in the ileal section were found in the aureomycin-supplemented calves.

P22. Results of feeding antioxidants to calves. K. E. Gardner and R. G. Hansen, Univ. of Illinois, Urbana.

Since some alfalfa dehydrators have proposed the use of antioxidants 6-ethoxy-1, 2-dihydro-2, 2, 4-trimethylquinoline (quinoline), and 2, 5-ditertiary butyl-hydroquinone (hydroquinone) at a rate of 0.065 g/lb of dried alfalfa, the toxicity of these compounds for young calves was of interest. Two levels of antioxidant providing 0.68 and 6.8 g/lb of total dry matter intake were fed to male calves of several breeds 1 to 16 wk. of age. They received alfalfa hay, a calf starter and small amounts of whole milk, which served as a carrier for the antioxidants.

At the 10 × level, growth exceeded the normal rate on 4 calves, 2 of which received quinoline and 2, hydroquinone. These calves were apparently not affected in any way.

On the 100 × level, feed intake was poor and 3 quinoline calves died in 6 to 22 d.; the 4th gained poorly and was removed from trial. One calf showed tissue quinoline levels of 160 p.p.m. of wet tissue for body fat, and 12.3, 7.1, 5.1, 2.2, and 1.6 for liver, skin, kidney, spleen, and muscle, respectively. Two calves on 100 × levels of hydroquinone ate poorly, made practically no gains, and were removed from trial at 60 d.

P23. Effects of tocopherol administration on dairy calves receiving various filled milks. R. S. Adams, T. W. Gullickson, J. H. Sautter, and J. E. Gander, Univ. of Minnesota, St. Paul.

When tocopherol was administered orally at the rate of 500 mg. daily, no significant differences were noted between alpha tocopherol acetate and free alpha tocopherol in respect to maintaining high blood plasma tocopherol values in calves receiving corn oil or lard filled milks. Intramuscular injections of alpha tocopherol acetate in sesame oil, however, failed to maintain blood plasma tocopherol values at levels approaching those obtained with oral administration. Both intramuscular and oral administration of either tocopherol supplement were successful in preventing the development of cardiac and skeletal muscle involvement in calves fed corn oil and lard-filled milks or a low-fat (fortified skimmilk) ration.

Additional findings on muscular dystrophy in animals receiving a filled milk containing oxidized butter oil will be reported. Moreover, the implications arising from the failure of the tocopherol-deficient low-fat diet to produce vitamin E deficiency symptoms under these experimental conditions will be discussed.

P24. Colostrum as a substitute for whole milk in a limited whole milk feeding system. W. S. Gaunya, R. D. Mochrie, H. D. Eaton, and R. E. Johnson, Univ. of Connecticut, Storrs.

Fifteen Jersey and Guernsey calves were used to determine the effects of colostrum as a substitute for whole milk in a limited whole milk feeding system. Calves were randomly assigned, with restriction as to breed, to 1 of 3 treatments as follows: *(a)* whole milk, *(b)* colostrum diluted with an equal amount of warm water, and *(c)* undiluted colostrum. The colostrum was derived from the 1st 6 milkings of all cows freshening during this period. The colostrum was pooled by cows and frozen in gallon containers. The average per cent total solids and per cent butterfat of the colostrum were $14.59 \pm 0.30\%$ and 3.61 ± 0.41. Milk or colostrum was fed at the rate of 7 lb. daily for the 2nd through 7th d., and 6 lb., 5 lb., 4 lb., and 2 lb., for the 2nd through 5th wk., respectively. Starter and U.S. No. 1 mixed hay chopped at a setting of ¼ in. were fed ad lib. from the 8th d. The mean adjusted weights at 35 d. for the 3 treatments were 79.7, 81.1, and 86.2 lb., respectively, with a standard deviation of 7.8 lb. The mean gains from birth to 35 d. were 18.0, 19.4, and 24.5 lb., respectively. These differences were not significant.

The incidence of scours was somewhat higher in the colostrum groups, the av. scour days being 2.6, 4.4, and 6.8, respectively, and the differences approached significance ($P < 0.10$). The av. scour days with one rectal temperature of 103° F. or more were 0.8, 1.8, and 1.2, respectively. These differences were not significant.

P25. The utilization of all the colostrum produced by a dairy herd for feeding the calves. E. A. Keyes, E. J. Peace, and J. L. Brence, Montana Agr. Expt. Sta., Bozeman.

Thirty-six Holstein calves were divided at birth and fed in 3 groups for 80 d. Group I was fed colostrum and whole milk intermittently, Group II was fed whole Holstein milk from different cows intermittently, and Group III was fed whole Holstein milk continuously from 1 cow. At the beginning of every 16-d. cycle, the calves were changed from their regular milk to 100% first milking colostrum. Colostrum was substituted for milk of equal weight in Group I with no scouring effect. The calves were weighed and TDN were computed on the milk, hay, and grain fed each calf every 4 d. There was no visible difference between the calves fed colostrum and those fed whole normal milk intermittently, the calves fed whole Holstein milk from several cows with frequent changes, or the calves fed whole Holstein milk from 1 cow continuously. There was no significant difference between any of the 3 groups of calves when TDN consumed were compared with gain in weight. Colostrum did not cause scours. The results indicate that equal weights of colostrum may be successfully substituted for milk in feeding young dairy calves, thus saving salable milk.

P26. Further studies on the use of soybean flour and whey solubles in milk replacement formulas for young dairy calves.[1] J. F. Stein

and C. B. KNODT, The Pennsylvania State Univ., State College.

Soybean flour and whey solubles when fed in a 7:1 ratio in dried processed form are more effective in replacing oat flour, dextrose, and dried brewers' yeast in the basic formula studied than the individual components per se. On the basis of the data obtained, a simplified formula (lb. basis) containing nonfat dry milk solids, 38.5; dicalcium phosphate, 2.5; trace minerals, 0.5; Aurofac D, 1; vitamin A and D_2 supplement (4,000 U.S.P. units A and 500 U.S.P. units D_2 per g.), 0.5; and combined dried form of 7 parts soybean flour to 1 part whey solubles, 57, has given mean daily body weight gains of 1.11 lb. to 49 d. of age.

[1] Support of The Borden Co. and Lederle Labs. is acknowledged.

P27. Effect on calves of a whole milk diet supplemented with known nutrients. J. W. THOMAS and M. OKAMOTO, Dairy Husb. Research Branch, USDA, Beltsville, Md.

In order to study further the fundamental nutritional requirements of calves over extended periods, whole milk has been used as a basal diet. This diet was supplement with adequate amounts of magnesium, iron, copper and vitamin D, which are known to be required by the calf on a whole milk diet. Manganese and cobalt also have been added.

The calves were kept on screen floors with occasional exercise in a yard on a cement slab. Calves on this diet have died when 5 mo. to 1 yr. of age. The following symptoms are usually found, although not all simultaneously in the same calf: anorexia, listlessness, excessive nervousness, mild diarrhea, hyperthomia, septicemia, uremia, hyperphosphatemia, hypercholesterolemia, anemia, a slight hypercalcemia, and upon autopsy an enlarged kidney and liver. Near the terminal stage, supplementation with numerous items such as alfafa, linseed meal or water extracts of both, liquid rumen contents, ascorbic acid, niacin, tocopherol, sulfa drugs, Aurofac, yeast, or sodium propionate have not alleviated the above mentioned symptoms, although tocopherol did prevent pale, white muscles.

It is apparent that the growing calf requires nutrients still unidentified. When calves are fed normally it is possible that these nutrients are present in the feeds or are synthesized in the rumen. A nutritional imbalance is another possibility which must be considered in calves fed whole milk exclusively.

P28. Relationship between certain corn products in the diet and the concentration of magnesium in the blood serum of dairy calves. G. E. HAWKINS, JR., Alabama Polytech. Inst., Auburn.

This study was initiated to provide information on the effect of certain corn products on the utilization of dietary magnesium and to develop a basal diet for studying the needs of dairy calves for dietary manganese.

Eighteen dairy calves were randomly assigned to test diets in which nonfat dry milk solids, hominy grits, and lard were the principal sources of energy. Corn products were incorporated in the concentrate mixture, as follows, by diets: A, corn gluten meal, 12.2%; B, corn hulls, 1.2%; C, corn gluten, 5%; D, corn oil, 1.5%; E, corn oil meal, 1.5%; and F, corn solubles, 3.0%. During the early phase of the study liquid skimmilk which was fed at the rate of 1 lb. per 10 lb. of body wt. was supplemented with magnesium sulfate to provide 0.21% magnesium on the dry basis, whereas the concentrate component of each diet which was fed in excess of the amount the calves would consume provided 0.14% magnesium on the dry basis. Since there were no significant changes in the concentrations of blood serum magnesium after the calves had been on the diets from 56 to 174 d., the magnesium supplement to the liquid skimmilk was discontinued, after which the entire diet of the calves provided 0.14% magnesium on the dry basis. Sixty d. after the magnesium supplement was discontinued, the mean concentrations of blood serum magnesium associated with diets were: A, 1.89; B, 1.41; C, 1.37; D, 1.39; E, 1.22; and F, 1.69, and the L.S.D. ($P = 0.05$) was 0.28 mg. per 100 ml.

P29. The effect of management and housing upon growth and health of dairy calves. W. R. MURLEY, E. W. CULVAHOUSE, and R. K. WAUGH, North Carolina State College, Raleigh.

Eighty-four female calves were randomly assigned within breeds to a conventional closed barn or an open shed in individual pens at 3 d. of age. At 8 wk. of age the calves in each type of barn were randomly sub-grouped; one half remained in the individual pens to 4 mo. of age and one half were turned to a dry lot for exercise during the days and returned to the pens at night. Whole milk was fed during the 1st 8 wk., and ad lib. feeding of hay and a maximum of 4 lb. per day of a commercial calf starter, with amounts eaten recorded, was initiated at 1 wk. of age. At 10 wk. a simple grain mixture replaced the calf starter in the ration. Temperature and relative humidity were recorded continuously throughout the trial. Group mean body weight changes were 0.88 and 0.85 lb. daily during the first 8 wk. for the calves in the closed barn and open shed, respectively, and 1.10 and 1.20 lb. during the succeeding 8 wk., although the differences were not significant statistically. The exercise had no apparent effect on the growth or appearance of the calves. The general health of the calves was not greatly different; however, 2 cases of pneu-

monia resulted in death in the closed barn. Fecal examination for parasites was negative throughout the trial.

P30. A comparison of cottonseed meal, urea, and ammoniated molasses (32%) in the ration of dairy heifers. J. B. FRYE, JR., B. T. PARHAM, B. L. KILPATRICK, and L. L. RUSOFF, Louisiana Agr. Expt. Sta., Baton Rouge.

Twenty-four Holstein and 9 Jersey heifers, 22-30 mo. of age, were grouped into 3 similar lots. In the concentrate mixture containing 12.5% crude protein, urea and ammoniated molasses replaced the cottonseed meal, which amounted to 30% of the protein. A Latin square design was used, comprising 7-d. preliminary periods and 28-d. experimental periods. Each animal was fed daily 25 lb. of corn and soybean silage, 3 lb. of chopped grass hay, and the grain mixture according to body weight, which averaged 7.2 lb. per animal. Three-day body weights were obtained at the beginning and end of each period.

A preliminary analysis of the data indicate that urea and ammoniated molasses (32%) are comparable to cottonseed meal in feeding value.

P31. The value of dicyanodiamide as a nitrogen extender in rations for dairy heifers.[1] N. D. MAGRUDER and C. B. KNODT, The Pennsylvania State Univ., State College.

A feeding trial was conducted to determine the value of dicyanodiamide as a nitrogen extender in the rations of dairy cattle. Dicyanodiamide, a crystalline compound, has approximately 66% nitrogen. In preliminary studies 1 unbred Holstein heifer was fed 10% dicyanodiamide in the grain ration for a 30-d. period without ill effect.

After a 30-d. preliminary feeding period, 12 unbred Holstein heifers were divided into 4 groups on a mean body weight per group basis. The grain rations fed for 180 d. differed as follows: Group I, basal (8.5% total protein); Group II, basal + soybean oil meal (16.4% total protein); Group III, basal + urea (16.6% total protein); and Group IV, basal + dicyanodiamide (16.6% total protein). Each supplement supplied approximately 47% of the total protein of the grain ration. At the conclusion of the feeding trial the average daily gains were 0.87, 1.07, 0.97, and 0.99 lb., respectively.

The digestibility coefficient for nitrogen of the dicyanodiamide ration was similar to that of the soybean oil meal and urea rations. Chemical analyses of feces collected at the 5th and 10th day of the digestion period compared favorably.

[1] The assistance of Lederle Laboratories is acknowledged.

P32. Further observations on the long-time effects of antibiotic feeding to dairy cattle. N. L. JACOBSON, J. T. MILES, and L. D. MCGILLIARD, Iowa State College, Ames.

One heifer in each of 17 pairs received approximately 240 mg. aureomycin (in Aurofac 2A) daily for a minimum of 15 mo. prior to parturition and during the first lactation. Age at first parturition was similar for both groups (26 mo.). Mean body weights 10 d. after calving were 995 lb. for the aureomycin-fed animals and 939 lb. for the controls. No differences between groups in milk or fat production were observed. The mean birth weights for calves from the aureomycin-fed and control dams, respectively, were 76 and 66 lb.

Ten calves from each of the 2 groups of dams were divided into 2 comparable sub-groups and were fed in accordance with a limited whole milk feeding plan. The calves in 1 sub-group from the aureomycin-fed dams and in 1 sub-group from the controls were fed aureomycin. The weight gain of these calves was 26% greater during the first 16 wk. than that of calves in the sub-groups receiving no antibiotic supplement. Weight gains were not affected appreciably by dam's diet. Starter consumption was greater and incidence of diarrhea was lower for calves fed the antibiotic.

P33. The effect on milk production of feeding alfalfa harvested and stored by four different methods. E. A. KEYES and E. P. SMITH, Montana Agr. Expt. Sta., Bozeman.

A feeding experiment using alfalfa hay harvested and stored by 4 different methods — chopped hay, baled hay, loose hay, and silage — was conducted. The experiment was undertaken to determine which kind was nutritionally the most valuable and which would produce the most milk per lb. of hay consumed during the feeding season. Twenty-eight Holstein and Jersey cows were used in the experiment. They were divided into 4 equal groups with each group receiving a different kind of roughage. All cows were fed the same grain ration containing 19.43% crude protein. The experiment revealed that all 4 kinds of roughage were adequate nutritionally for body maintenance. All the cows gained in weight throughout the 120-d. experimental period. However, more milk was produced by the silage-fed cows per lb. of protein, TDN, and total roughage consumed.

P34. The effect on lactating dairy cows of replacing hay with corn silage. R. K. WAUGH, W. R. MURLEY, H. A. POSTON, and R. D. MOCHRIE, North Carolina State College, Raleigh.

A ration with corn silage as the sole roughage was compared with similar rations containing varying amounts of hay for lactating dairy cows. The experiment consisted of 6 continuous trials at 3 locations during 2 winter feeding

periods using 60 lactating dairy cows of the Ayrshire, Guernsey, Holstein, and Jersey breeds. The trials consisted of a 28-d. standardization period and a subsequent 56-d. experimental period. A concentrate mixture was fed at the rate of 1 lb. for each 4 lb. FCM daily, using the standardization period as the base and reducing the amount 5% each 4 wk. Although not statistically different, average daily FCM adjusted for initial production was 30.4, 31.4, 29.8, and 29.2 lb., respectively, for cows receiving 1.0, 0.5, 0.25, and 0.0 lb. of hay per 100 lb. of body weight in addition to corn silage ad lib. Mean dry matter, adjusted for initial intake, was 25.5, 25.0, 22.7, and 21.8 for the above groups, respectively, a difference that was significant statistically. The dry matter received from silage increased from an average of 9.3 lb. daily for all cows during the standardization period to 11.2, 13.2, 13.5, and 14.1 lb. daily for the above groups, respectively. Differences in body weight changes were not significant statistically.

P35. Lima bean silage for dairy cattle. C. R. RICHARDS, Univ. of Delaware, Newark.

The feeding value of lima bean silage has not been reported. Lactation studies were made in 1952-53 and 1953-54 with 6 Holstein cows in a single reversal experimental design with feeding periods 6 wk. in length. Each group (lima bean silage Group I, corn silage Group II) was fed 30 lb. of silage daily, hay ad lib., and concentrates according to production except for 1.5 lb. additional concentrates per day for the lima bean silage-fed group in 1953-54 in an attempt to compensate, in part, for the grain in the corn silage. Feed intakes for Groups I and II, respectively, were: silages, 25.9, 28.1; hays, 28.4, 24.2; concentrates, 14.7, 14.0. Production of FCM was 40.2 and 40.7 lb. per day for I and II, respectively. Estimated TDN intakes (Morrison's table of feed composition and an arbitrary 14% TDN in lima bean silage) for I and II were 28.9 and 27.7, respectively. The composition of lima bean silages in 1952-53 and 1953-54 were: dry matter, 25.1, 23.0; crude protein, 3.2, 2.8; ether extract, 0.9, 0.7; ash, 4.9, 2.4; carbohydrates, 16.1, 17.1 (crude fiber 7.8, 8.4), respectively.

P36. Composition and nutrient value of grass silages throughout Vermont. J. A. NEWLANDER, Univ. of Vermont, Burlington.

The dairy farmers in Vermont have the privilege of sending silage samples to the Expt. Station for fodder analyses. The author has divided 154 grass silage samples thus received and analyzed into 5 arbitrary groups based on dry matter content. TDN values were then assigned to each group using digestion coefficients obtained at this station and from Morrison's *Feeds and Feeding*, 21st ed.

	D.M.	No. samples	D.M. range	D.M. Av.	Av. TDN
	(%)		(%)	(%)	(%)
Group 1	-20	9	15.69-19.85	18.86	9.44
2	20-25	45	20.40-25.95	23.28	12.82
3	25-27	36	25.08-26.89	25.95	15.06
4	27-30	29	27.01-29.98	28.59	16.56
5	30-40	35	30.00-42.03	34.37	19.95

Thus if 40 lb. of silage from Group 5 meets a certain food requirement, 48 lb. must be fed from Group 4; 53 from Group 3; 62 from Group 2; and 85 from Group 1 to provide the same amount of nutrients. The question might be raised, Why harvest so much water?

P37. Effect of added ground corn on the composition and palatability of stacked legume-grass silage. R. S. ALLEN and A. R. PORTER, Iowa State College, Ames.

Approximately 450 T. of chopped, non-wilted forage (mostly alfalfa and brome grass; mean moisture content, 76.4%) was ensiled in a stack on a concrete slab (24 × 100 ft.). Ground corn (100 lb. per T.) was added to half of the material (12 × 100 ft. section) at the time of ensiling. Samples for chemical analyses were taken at the time of ensiling and approximately 22 and 31 wk. later. The silage samples from each half of the stack were obtained at approximately 1, 3, and 5 ft. below the top spoilage area (about 8 in.) and 2.5 ft. on each side of the center sample. On a dry matter basis, silage with added corn was higher in acetic acid, linoleic acid, and total acidity, whereas silage without added preservative contained more linolenic acid, butyric acid, and carotene. All constituents measured, except for dry matter, tended to increase with increasing depth of silage. Cows showed a distinct preference for the corn-preserved silage when the diet was restricted to silage alone or to silage plus hay. Addition of concentrates on the silages in the feed bunks erased any preference pattern, and total consumption was increased.

P38. Ensiling succulent pasture forages with dried citrus pulp. R. B. BECKER, J. M. WING, P. T. DIX ARNOLD, G. K. DAVIS, and J. T. MCCALL, Florida Agr. Expt. Sta., Gainesville.

Pit silos (4 × 8 ft.) were filled with unchopped green oats, Pangola grass, and a mixture of White Dutch clover and Dallis grass. Additions of 0, 150, and 250 lb. per ton of dried citrus pulp were used with each forage in separate silos. The silos held 1,420 to 2,578 lb. of these forages. Sound silage amounted to 87% or more of materials weighed in. Surface and edge spoilage averaged 3%, ranging from 0 to 4.5%. Muslin bags containing dried citrus pulp placed in the bottom of 3 silos gained 68 to 89% in weight.

Consumption of plain Pangola silage averaged 35 lb. daily per Jersey cow, as compared with 38 and 42 lb. for the Pangola-citrus pulp

silages. As much as 60 lb. of clover-citrus pulp silage were eaten daily per cow.

In general, there was about 90% preservation of protein and 86% of nitrogen-free extract, with little change in ash and crude fiber, and a slight increase of ether extract (crude fat) in the finished silages.

The main change evident in the bagged citrus pulp placed in the bottoms of 3 silos appeared to be from absorption of nitrogen-free extract from the silage juices.

P39. The use of acidifying salts in high moisture hay crop silage. C. H. GORDON, H. G. WISEMAN, L. E. CAMPBELL,[1] C. G. MELIN, and H. M. IRVIN, Dairy Husb. Research Branch, U. S. D. A., Beltsville, Md.

A 1953 first cutting grass-clover mixture was harvested at an early hay stage without wilting and stored in 3 concrete silos. The average moisture content of the stored material was about 81%. The treatments used were: 8.2 lb. sodium meta-bisulfite per ton, 5.1 lb. of Kylage (a patented product consisting mainly of calcium formate and sodium nitrite) per ton and no additive.

Nutrient losses in the three silages were measured by input-output weights and analysis. Dry matter digestibility also was determined. The resulting silages showed both additives to be effective in lowering pH, retarding protein breakdown, improving odor, and preserving carotene and sugar. Feed values and palatability were measured by a feeding trial in which the silages were the sole roughage in the rations of 3 groups of milking cows. Results indicate these acidifying treatments to be effective in improving the type of silage often produced from untreated high moisture hay crops. The heavy losses and inconvenience from large amounts of seepage continued to be an acute problem.

[1] Agricultural Engineering Research Branch, Farm Electrification Section.

P40. Some chemical characteristics of grass silage made with sodium meta-bisulfite.[1] G. ALDERMAN, R. L. COWAN, J. W. BRATZLER, and R. W. SWIFT, The Pennsylvania State Univ., State College.

During 1953 a large number of silos on farms in Pennsylvania were filled with a variety of unwilted grass forages treated with sodium meta-bisulfite at the rate of 8-10 lb/ton of green forage. Samples of these silages were collected, when fed, and were analyzed subsequently to determine the nature and extent of the fermentation which had occurred. Bisulfite-treated silages showed a limited degree of fermentation with the formation, on the dry matter basis, of approximately 5% of lactic acid and less than 2% of acetic acid. Butyric acid was either present in negligible amounts or absent. The pH values ranged from 4.1 to 4.9, and residual sulfite was equivalent to approximately 20% of the bisulfite added originally. Ammonia nitrogen was low in all samples, though considerable hydrolysis of proteins to amino acids was evident. Varying amounts of reducing sugars were found with an average of 2.5% (as glucose). The amounts of fermentation products were independent of the type of grass forage ensiled and were less than the amounts found in silages made by the use of added carbohydrates. The almost complete absence of butyric acid in these silages made from unwilted forages appears to indicate that sodium bisulfite inhibits butyric acid formation while allowing lactic acid formation to proceed to a limited extent.

[1] This research was supported in part by the award of an International Fellowship to G. Alderman by the W. K. Kellogg Foundation, Battle Creek, Mich., and by grants from the Monsanto Chemical Co., Everett, Mass., the General Chemical Division, Allied Chemical and Dye Corp., New York, and the New Holland Machine Div., New Holland, Pa.

P41. Field trials with sodium meta-bisulfite grass silage. J. W. BRATZLER, R. L. COWAN, and R. W. SWIFT, The Pennsylvania State Univ., State College.[1]

During 1953 approximately 50 Pennsylvania farmers cooperated in testing the efficacy of sodium meta-bisulfite as a preservative for grass silage under actual farm conditions. A variety of forage crops were ensiled which included orchard grass, brome grass, timothy, alfalfa, red clover, sweet clover, and ladino clover. Reports from the farmers revealed their unanimous approval of sodium bisulfite as a preservative for grass silage when applied at the recommended rate of 8 lb/ton of green, unwilted forage. The advantages were *(a)* a silage entirely free of obnoxious odors, *(b)* superior palatability of the silage, *(c)* ease with which the preservative could be applied to the fresh forage, and *(d)* cheapness of the preservative. Results of the field trials, together with less detailed reports from hundreds of additional farmers who used bisulfite for the first time, served to emphasize the dissatisfaction that farmers have held for making grass silage without the addition of any preservative or with preservatives that have been in use during the past decade.

[1] This research was supported in part by the Monsanto Chemical Co., Everett, Mass., the General Chemical Div., Allied Chemical & Dye Corp., New York, and the New Holland Machine Div., New Holland, Pa.

P42. Using identical twin dairy cows to determine the effect of iodinated casein (Protamone) on milk production, thyroid activity, and body weight changes. E. W. SWANSON, R. A. MONROE, and C. L. COMAR, Univ. of Tennessee, Knoxville.

When both heifers of a pair of identical twins were fed 15 g. Protamone daily, their responses during a 13-wk. treatment and 30 d. after treatment were identical. One heifer of each 2 other sets was treated while the mate was the control. Production increases at the 3rd to 5th wk. were 60 lb. for one and 42 lb. for the other, while their mates continued to decline. Butterfat tests increased 1.0%. Thyroid activity was determined by measuring the uptake of radioactive iodine by the thyroid gland. Before feeding Protamone the twins' uptake of I^{131} was nearly identical. The thyroid of the Protamone-fed calf absorbed only an insignificant amount of I^{131} during treatment; but by 3 wk. after treatment was stopped, her thyroid uptake was again equal to her mate's. Respiration and heart rates of the treated twins were above their controls, but their body temperatures were higher only when the barn temperature exceeded 80° F. The treated heifers lost an average of 34 lb. in 5 wk., then gained weight at the same rate as the controls until Protamone was stopped. In the next 3 wk. the treated heifers averaged 90 lb. gain compared to 34 lb. for the controls. Body water content averaged 72% in controls, 69% in Protamone-fed cows, and 64% after Protamone withdrawal. The terminal increase in weight was not water but more likely was an increase in stomach and intestinal fill. This was evidenced by an increase in paunch girth of 6.1 in. during the rapid weight gains while the controls increased only 2.0 in.

P43. Results with identical twins in the feeding of thyroprotein. J. W. Thomas and M. Okamoto, Dairy Husb. Research Branch, U. S. D. A., Beltsville, Md.

Three sets of identical twin Holstein cows and 1 set of triplets have completed their first lactation, during which 1 member was fed TDN at 125% of Morrison's maximum requirement from the 50th to the 310th day of lactation and the other was fed TDN at the same rate plus thyroprotein at 12 g. per day. In 3 sets of animals the control cow produced the most milk (1,000 lb. more FCM), and in 1 set the cow receiving thyroprotein outproduced her control by 1,000 lb. Near the end of the lactation all 4 cows fed thyroprotein weighed less than their pair mates, averaging 170 lb. less with a range of 50 to 250 lb.

In the set of triplets the 3rd member was fed TDN at 100% of Morrison's maximum requirement without thyroprotein. This animal produced 9,000 lb. of FCM; the other control cow fed TDN at 125% produced 11,190 lb., and the one fed thyroprotein and TDN at 125% produced the intermediate amount of about 10,000 lb.

These results with identical twins confirm previous results when thyroprotein was fed under similar conditions to unrelated animals.

P44. The effect of feeding different amounts of potassium iodide on the level of protein-bound iodine in cows. J. F. Long, J. W. Hibbs, and L. O. Gilmore, Ohio Agr. Expt. Sta., Wooster.

Graduated levels of KI were fed to 2 mature Jersey cows in order to determine the extent to which the amount of KI in the ration might influence the PBI level in the blood. The amount of KI added to the ration daily was increased at weekly intervals from 11 mg. to 2.816 g., over a 14-wk. period. The predosage serum inorganic iodine level of 2γ% was maintained at both the 11 and 22 mg/d KI feeding level. At the 44 mg/d feeding level the serum inorganic iodine rose to about 10γ% and increased rapidly to 495γ% at the highest level of KI feeding.

The serum PBI level first showed a definite increase at the 44 mg/d KI feeding level over the predosage level of 3.5γ% and 6.4γ% for the two cows to 5.2γ% and 8.5γ%, respectively. PBI rapidly increased from this point to an average of about 72γ% at the highest level of KI feeding.

No clinical evidence of hyperthyroidism could be detected even at the highest levels of PBI, indicating that the PBI resulting from the KI feeding was noncalorigenic. This probably accounted for a large proportion of the PBI previously found in thyroprotein-fed cattle, which also had extremely high inorganic iodine levels. These results indicate that KI ingested in the small amounts usually found in rations containing iodized salt would not influence PBI values unless the amount exceeds 22 mg/d. Making inorganic iodine determinations along with the PBI offers a way for detecting samples in which high PBI values may be due to a high inorganic iodine intake and thus not be a reliable indicator of thyroid status.

P45. The effect of restricted roughage, high-concentrate diet upon milk fat, blood glucose and blood ketones. P. J. Van Soest, N. N. Allen, and L. R. Maki, Univ. of Wisconsin, Madison.

Studies were made on 6 lactating cows fed on normal and restricted roughage, the latter consisting of 3 lb. hay daily plus concentrates ad lib. The milk fat declined from an average of 4.62% on normal feeding to 2.14% on restricted roughage. Glucose and ketone determinations were made on 82 blood samples taken throughout the study. Blood ketones showed a significant decline from an average of 4.62 mg.% on normal feeding to 2.26 mg.% on restricted roughage. Blood glucose showed a tendency to increase; however, this was nonsignificant. Correlation of the milk fat with blood ketones was + 0.400; of ketones with glucose, –0.326; both highly significant. The correlation between glucose and milk fat was not significant.

The oral administration of 1 lb. sodium acetate $3H_2O$ daily to fat depressed cows caused

recoveries in fat test amounting to as much as 1.6%. There was a variable time lag in recovery after acetate feeding started, but all showed a marked recovery after 4-5 days of feeding. When acetate feeding was stopped, the test returned in a few days to the subnormal level. Propionate and carbonate feeding on an equivalent basis had comparatively little effect on the milk fat.

P46. Studies on the etiology and treatment of ketosis in dairy cows. J. C. SHAW, R. E. BROWN, R. A. GESSERT, and A. C. CHUNG, Univ. of Maryland, College Park.

The intramuscular injection of 200 to 600 AVU of ACTH in gel results in a marked increase in blood sugar and a sharp decrease in blood eosinophils within 24 hr. in both normal and ketotic cows. A marked decrease in milk production also occurs within 24 hr. The effect upon blood sugar and milk production is usually somewhat greater with normal cows. Similar effects were produced by the injection of 1 g. of hydrocortisone alcohol, but not with hydrocortisone acetate or cortisone acetate. Although as little as 0.5 g. of hydrocortisone alcohol produced a 2- to 3-fold increase in blood sugar in ketotic cows within 24 hr., the clinical response has been variable. From 1 to 1.5 g. of hydrocortisone acetate was somewhat more effective clinically, but the blood glucose response was slower. A combination of the alcohol and acetate form of hydrocortisone appeared to be much more effective than either one alone. Treatment of ketotic cows with intramuscular injections of 4 g. of 11-ketoprogesterone usually resulted in rather marked clinical improvement within 24 hr. However, the blood sugar did not increase markedly until the 2nd or 3rd d. after treatment. The differences appear to be due to differences in the relative rates of absorption and/or utilization. The intravenous injection of 250 g. of glucose usually will not maintain the blood glucose of normal cows above normal more than 90 to 120 min. A combination of 250 g. of glucose and 50 USP units of ACTH given intravenously maintains blood sugar above normal for 16 to 24 hr. Studies up to date made on cows during the development of ketosis have failed to disclose any marked alterations from the controls in the levels and relative proportions of acetic, propionic, and butyric acids. Dissimilation tests on various substrates by washed cell suspensions also have failed to disclose any pattern different from the controls.

P47. The influence of intravenously injected calcium borogluconate on the blood levels and urinary excretion of calcium in dairy cows with parturient paresis.[1] J. L. ALBRIGHT and T. H. BLOSSER, State College of Washington, Pullman.

[1] This study was supported in part by the Jensen-Salsbery Laboratories, Inc., Kansas City, Mo.

A study was undertaken to compare a 32.7% calcium borogluconate solution with one containing 25% calcium borogluconate when injected at the rate of 0.5 ml/lb body weight into milk fever–afflicted and normal animals. The purpose of this experiment was to study the physiological significance of differing blood and urinary excretion levels in normal and parturient paresis cows.

Seven cows that were treated with the 32% solution had blood samples taken immediately pretreatment, 15 min. post-treatment, every 2 hr. for a 24-hr. period, 36, 48, and 72 hr. post-treatment. Blood calcium levels for these times were: 4.7, 25.7, 17.0, 13.7, 12.2, 10.4, 9.8, 9.6, 8.9, 8.1, 7.7, 7.6, 7.6, 7.1, 7.5, 9.7, and 12.2 mg.%, respectively. The corresponding blood levels for 5 animals treated with the 25% solution were: 5.7, 21.6, 15.5, 13.8, 11.2, 9.9, 9.2, 8.8, 8.7, 8.1, 7.8, 7.3, 6.4, 6.7, 8.2, 8.7, and 10.2 mg. %, respectively. There were 5 relapses out of 9 cows treated for milk fever with the 32% solution, whereas with the 25% solution 3 relapses were observed from treatment of 5 animals. Fourteen milk fever attacks were observed in 10 animals in this study.

Two of 3 normal parturient cows injected 12 hr. postpartum with the 32% solution (av. pre-injection Ca level, 9.9 mg. %) developed parturient paresis symptoms 48 hr. postcalving, whereas 2 normal parturient animals injected 12 hr. postpartum with the 25% solution reacted normally. Three first calf heifers were injected with the 32% solution but did not develop symptoms of parturient paresis.

P48. Availability of phosphorus from phosphates after irradiation in the pile. L. R. ARRINGTON, J. C. OUTLER, and G. K. DAVIS, Florida Agr. Expt. Sta., Gainesville.

Phosphate materials which had been irradiated in the pile were fed to growing calves and rats to determine the availability of phosphorus from different sources. Animals were maintained in metabolism cages for periodic excreta collections and were sacrificed at 144 hr. (calves) and at 72 hr. (rats) to determine tissue distribution of phosphorus. In order to account for phosphorus which is absorbed and re-excreted into the gastrointestinal tract, 3 calves were given an intravenous dose of radioactive phosphorus, and the percentage of the dose which was excreted into the feces and gastrointestinal tract was determined.

When activated defluorinated rock phosphate was administered orally to calves, 15% of the dose was excreted in the urine and 31% in the feces by 144 hr. Total rat excretions by 72 hr. were: urine 10%, feces 45% from rock phosphate and 8 and 12% from phosphoric acid. Radiophosphorus administered intravenously to calves was excreted as follows by 144 hr.: urine 0.3%, feces 8%, and contents of gastrointestinal tract 3.5%. Studies with $CaHPO_4$ are being continued.

P49. The modified Richardson Biochemical Pregnancy Test as applied to dairy cattle. SID DIAMOND, S. H. MORRISON, and J. D. DONKER, Univ. of Georgia, Athens.

The purpose of this study was to determine whether the Richardson Test, which is a biochemical test for pregnancy in humans and reported to be highly dependable, simple to perform, and requiring a minimum of time, equipment, and expense, is adaptable to the bovine female.

The product of pregnancy which is presumably identified by the Richardson Test is free estrone. The reaction utilizes 2, 4-dinitrophenylhydrozine in 95% ethanol to form a brown colored hydrazone with the estrone of pregnancy.

Beginning in Nov., 1952, and ending July, 1953, urine samples were collected monthly from 75 cows, open and in various stages of pregnancy. These were tested by the modified Richardson procedure. There were 623 tests carried out on Holstein and Jersey cows.

In 400 trials on known pregnant cows there were 194 instances of negative reactions and on 147 trials on open cows there were 65 positive reactions. This test as applied in its present form to the bovine is therefore useless for predicting pregnancy. It appears from the above data that the observed positive and negative reactions are not associated with pregnancy.

P50. Changes in bovine blood proteins associated with parturition. B. L. LARSON, G. D. ROLLERI, and K. A. KENDALL, Univ. of Illinois, Urbana.

Blood serum was obtained at intervals prior to parturition and blood serum, colostrum, and milk after parturition to determine by a quantitative electrophoretic analysis the absolute changes of the individual proteins present.

The results indicated that the total blood serum protein level drops quickly a few days before, reaches a low on about the day of parturition, and rebuilds again rather rapidly. This drop, accounting for 10 to 25% of the total blood serum proteins, was due to the decrease in the β_2- and γ_1-globulins, since little changes were noted in the serum albumin, total α-, β_1-, or γ_2-globulin contents. The total amount of globulins leaving the blood at this time, however, did not always account for all of the immune globulins appearing in the colostrum. Electrophoretic analyses of the normal milk suggests the constant secretion of a low immune globulin level. This secretion during the period before parturition must be considered in order to account for the total immune globulins appearing in the colostrum.

Studies in progress on the level of production of the specific milk proteins reveal that some are in fairly constant production whereas others vary considerably during the lactation period.

P51. The isolation and identification of the causative agent of bovine hyperkeratosis (X-disease) from a processed wheat concentrate.[1] W. HANSEL, College of Agr., and P. OLAFSON and K. MCENTEE, N. Y. State Vet. College, Ithaca, N. Y.

In previous work the causative agent of hyperkeratosis was isolated from a wood preservative produced in Germany and identified as a highly chlorinated naphthalene. Similar procedures have been used to isolate the causative agent from a processed wheat concentrate. Ultra violet absorption curves, infra-red absorption curves, and the results of micro-analyses strongly indicate that the causative agent in the processed wheat concentrate is also a highly chlorinated naphthalene. Details of the isolation procedure will be presented.

[1] The work described was done in cooperation with the Bureau of Animal Industry, U.S.D.A., under interregional agricultural experiment station research on the project entitled, "X-Disease (Hyperkeratosis) of Cattle."

P52. Polysaccharide synthesis and lactic acid production by bovine rumen bacteria. R. N. DOETSCH and R. Q. ROBINSON, Univ. of Maryland, College Park.

Lactic acid production by mixed suspensions of bovine rumen bacteria is governed by a number of definite conditions. Among them are nature and concentration of substrate, length of incubation time, pH, and presence of chelating agents. During the course of experiments designed to determine whether lactic acid is a "normal" end-product of bovine rumen bacterial fermentations this compound has been found in suspensions from animals on various diets.

An iodine-staining substance is also found in bovine rumen bacteria, and in this case a set of conditions similar to those above must be met for its production. This polysaccharide material is believed to be a reserve substance since it disappears from cells in the absence of utilizable substrates.

The possible significance of these reactions to the physiology of the bovine ruminant will be emphasized.

P53. Studies on methane formation by washed suspensions of bovine rumen bacteria. J. J. MCNEILL and R. E. BROWN, Univ. of Maryland, College Park.

Methane gas is present in the bovine rumen in a quantity which comprises about 10% of the total energy value of the digestible nutrients in the animal's diet. Studies on the formation of methane were begun in order to obtain information concerning the role of rumen microorganisms in the production of this gas.

The experiments reported here were short-time fermentation studies with nonproliferating resting cells and were designed to show the substrates from which methane is produced and

the effect of different gaseous atmospheres upon methane production.

Glucose, maltose, cellobiose, pyruvate, lactate, propionate, acetate, formate, succinate, and fumarate were used as substrates. These compounds were incubated in the presence of rumen bacteria under anaerobic conditions with nitrogen, hydrogen, and carbon dioxide atmospheres. Various combinations of these gases also were used. After the incubation period, quantitative determinations were made for methane, carbon dioxide, hydrogen, and for total gas evolution and uptake with a modified Haldane gas analysis apparatus.

The formation of methane depends upon the nature of the substrate used and the gaseous atmosphere used to maintain anaerobiosis. Hydrogen gas has a pronounced effect on methane production and formate yields much higher quantities of methane than any other substrate.

P54. Evidence for an unidentified factor required by a rumen bacterium. R. H. Wasserman, H. W. Seeley, and J. K. Loosli, Cornell Univ., Ithaca, N. Y.

Studies of the microflora of mature ruminants receiving urea, molasses, and timothy hay resulted in the isolation of a number of anaerobic lacto-bacilli in dilutions of 10^{-7} to 10^{-10}. These isolated rumen bacteria were found to have relatively simple nutritional requirements. Some of the isolates could grow in a medium containing ammonium chloride, cysteine, phosphate-bicarbonate buffer, complex salts, purines, pyrimidines, and vitamins but were stimulated by the presence of crude natural materials. Other isolates had an absolute requirement for crude materials. The addition of 20 crystalline amino acids and/or crystalline vitamins did not replace the stimulating factor in the crude materials. Investigations were initiated in an attempt to gain an insight into the general properties of the growth factor and its distribution in natural materials. Potent sources of the factor were tomato juice serum, grass juice, distillers' dried molasses solubles, bovine feces, and others. Less potent sources were beef extract, tryptone, and extracts of soybean oil meal, corn, and rumen liquor. Relatively inactive sources were antipernicious anemia injectable liver extracts and timothy hay extracts. Tomato juice ash was completely inactive. The chromatographic adsorption spectra, chemical reactions, and physical properties were determined. Paper chromatographic separations indicated the multiple nature of the unidentified factor. Its possible relationship to other growth factors and the general importance of this factor to bovine nutrition will be discussed.

P55. The effect of washed suspensions of bovine rumen bacteria on the potency of aureomycin and terramycin. P. Jurtshuk, Jr., R. N. Doetsch, and J. C. Shaw, Univ. of Maryland, College Park.

The effect of mixed suspensions of bovine rumen bacteria on the activity of aureomycin and terramycin was studied. Various concentrations of these antibiotics were added to both heated (80° C. for 15 min.) and nonheated suspensions of rumen bacteria, and after incubation the potency was measured. Activity was determined by means of sensitivity tests (assay cup technique and turbidimetric methods) using *Micrococcus pyogenes* var. *aureus*. In addition, the effect of aureomycin and terramycin on the utilization of xylose, arabinose, glucose, maltose, and cellobiose was noted by determining the ability of rumen bacteria to ferment these substrates in the presence of antibiotic by measuring the time required for disappearance of reducing sugars.

No significant difference was observed between heated and nonheated cell suspensions in their ability to inactivate either aureomycin or terramycin. This is presumed to mean that a specific heat-labile inhibitor is not present. There was, however, a nonspecific inhibition of aureomycin and terramycin most likely the result of the protein of the cell suspensions. The ability of washed suspensions of bovine rumen bacteria to utilize the above-mentioned carbohydrates was definitely decreased by the antibiotics at the levels employed in this work.

P56. The effect of aureomycin feeding to dairy cattle on the in vitro digestion of cellulose by the rumen microorganisms. J. R. Lodge, J. T. Miles, and N. L. Jacobson, Iowa State College, Ames.

The effect of aureomycin feeding (Aurofac 2A) on the ability of rumen microorganisms to digest cellulose was studied by an artificial rumen technique. Two samples of rumen microorganisms were obtained from each of 20 cows, 10 of which had received 240 mg. of aureomycin per head daily from an early age. The inoculums from the aureomycin-fed cows consistently digested a lower percentage of cellulose (72%) than did those from the control cows (83%). Samples of microorganisms from calves 4 mo. of age receiving 80 mg. aureomycin per head daily digested 58% of the cellulose compared to 78% digestion by inoculums from control calves.

Aureomycin levels in the rumen liquid were 0.08γ/ml, 16 hr., and 0.22γ/ml, 4 hr. after feeding 240 mg. of the antibiotic. The level in the calves was 0.22γ/ml approximately 2 hr. after feeding 40 mg. of aureomycin (½ of the daily allowance).

The addition of 1.6γ of aureomycin per ml. of fermentation mixture in the artificial rumen severely inhibited cellulose digestion by the microorganisms from the control cows. The same addition had very little effect on the digestion of cellulose by the inoculums from the aureomycin cows.

P57. The influence of the ration and early rumen development on the volatile fatty acid content of rumen juice and blood sugar levels in high roughage fed calves. H. R. CONRAD, J. W. HIBBS, and W. D. POUNDEN, Ohio Agr. Expt. Sta., Wooster.

Total steam volatile fatty acids in rumen juice and blood sugar were measured periodically from birth to 12 wk. in calves raised on the high roughage system. Either alfalfa or clover-timothy hay was fed with different proportions of hay to grain. The volatile fatty acid content of rumen juice increased from an average of 60 meq/l at 4 wk. of age to a maximum level of 92 meq/l at approximately 9 wk. of age. A somewhat higher level was maintained when alfalfa hay was fed. The proportion of hay to grain fed did not significantly influence the total steam volatile fatty acid content of the rumen juice. The blood sugar level declined from 80-100 mg/100 ml. to 45-65 mg/100 ml. during the first 8 wk. and maintained this level through 12 wk. Calves fed all milk with no roughage remained at the 70-85 mg/100 ml. range to 12 wk.

The relative amounts of acetic, propionic and butyric acids at 12 wk. of age in calves fed a 2:1 ratio of alfalfa hay to grain were 60% (3.01 g/l), 24% (1.25 g/l) and 16% (0.83 g/l), respectively. A lower percentage of propionic and butyric acid was found when clover-timothy hay was fed. Rumen juice from 18-wk. old calves on ladino clover pasture was found to contain 58% (2.25 g/l) acetic, 19% (0.75 g/l) propionic, and 23% (0.90 g/l) butyric acid. The rumen juice of calves fed on grass silage using a 2:1 ratio of silage dry matter to grain contained 52% (3.11 g/l) acetic, 25% (1.46 g/l) propionic, and 23% (1.38 g/l) butyric acid.

P58. Volatile fatty acids in the rumen of cows with ketosis. L. H. SCHULTZ, Cornell Univ., Ithaca, N. Y.

Rumen samples were taken from 9 cows at the time of ketosis. After treatment and recovery, samples were again taken on the same cows, once on a winter ration and once on pasture. Analyses of rumen liquor for volatile-soluble fatty acids gave the following mean values, expressed in the order of ketosis, winter normal, and summer normal: total acids in mg/100 ml. — 448,617,627; % acetic — 60.7, 59.2, 54.6; % propionic — 17.8, 19.9, 21.1; % butyric — 21.5, 20.9, 24.3. Comparing ketosis to normal, total acids were lower ($P < 0.01$), the percentage of acetic was higher ($P < 0.05$), and the percentage of propionic was lower ($P < 0.05$), with no significant change in the percentage of butyric.

Since the above trends appeared to be related to feed intake, 6 of the cows were sampled before and after fasting for 16 hr., with the following results expressed in the order of normal and fasted: total acids in mg/100 ml. — 595,393; % acetic — 55.1, 58.7; % propionic — 21.7, 18.2; % butyric — 23.2, 23.1. Comparing fasting to normal, the total acids were lower ($P < 0.05$), the percentage of acetic was higher (although not significant), the percentage of propionic was lower ($P < 0.01$), and there was practically no change in the percentage of butyric. The rumen fatty acid picture after fasting, therefore, was similar to that at the time of ketosis.

P59. Yields and composition of holocellulose prepared from various feeds and feces. R. E. ELY and L. A. MOORE, Dairy Husb. Research Branch, USDA, Beltsville, Md.

Crude holocellulose was prepared from 10 forages by acid chlorite treatment. Yields of holocellulose ranged from 39 to 75% of the dry matter of the feeds studied. The ash, protein, and lignin-free holocellulose yields represented from 97 to 103% recovery of the calculated theoretical holocellulose contents of the 10 feed samples. Summation of the ash, protein, lignin, and holocellulose contents of the extracted feeds accounted for 98 to 103% of the dry matter. From 13 to 32% of the feed protein ($N \times 6.25$) is retained in crude holocellulose preparations with the method used. Pretreatment of the feed sample with pepsin reduces the protein content of crude holocellulose preparations and also removes a portion of the holocellulose fraction. Calculated digestibility coefficients of holocellulose from the feeds studied ranged from 50 to 83%.

The apparent lignin content of crude holocellulose prepared from feces of animals fed the 10 feeds was higher than in the crude holocelluloses prepared from the corresponding feed. The protein ($N \times 6.25$) content of crude holocellulose prepared from 10 feces samples was lower in 7 cases and higher in 3 cases than the crude holocellulose material prepared from the corresponding feed.

P60. Alfalfa–orchard grass grazing results. R. H. LUSH, A. G. VAN HORN, W. M. WHITAKER, and B. T. THROOP, Univ. of Tennessee and Dairy Husb. Research Branch, USDA, Knoxville.

Four-yr. (1950-1953) results indicate alfalfa may be maintained under rotational grazing with only a 20-d. interval for recovery, under a delayed or all season grazing in middle Tennessee. Four fields, each subdivided in 3 small fields for moderate grazing with Jersey cows for 10 d. each were used. Seeding and top dressing with potash borax were similar on all areas. The 2 fields grazed all season produced an average of 137 standard cow-days and 5,886 lb. of FCM per acre; the 2 comparable fields from which a 1st cutting of 3,024 lb. hay or 9,073 lb. silage was cut and then grazed produced an average of 84 cow-days grazing and

3,983 lb. of FCM per acre. The all season system produced the greatest net returns at lower labor cost, but the delayed system maintained a better stand of alfalfa and more estimated TDN from grazing. Chemical analysis of vegetation in June and October showed little variation between plots with rather high protein, low lignin, and average ash content. Rainfall averaged 5.8 in. below normal for the grazing season, which adversely affected yields and clover.

P61. Continuous vs. rotational grazing. A. D. PRATT and R. R. DAVIS, Ohio Agr. Expt. Sta., Wooster.

Comparison of continuous and rotational grazing for 3 pasture seasons of brome-alfalfa-ladino meadows has given about 20% greater returns of milk per acre from rotational grazing. A randomized block design replicated 3 times was used. The cows were fed different grain mixtures and hay individually in the stall. The rotation system consisted in moving the cows from 1 of 6 half-acre lots to another when the forage had been adequately grazed and clipping the lot from which they were moved. The continuously grazed lots remained unclipped throughout the season. Rotational grazing provided opportunity for using accumulated forage, not needed for pasture, as silage or hay. Under drought conditions accumulated forage provided pasture and maintained milk flow. Alfalfa was maintained in the stand for 3 or 4 yr. when grazing was from May 5 to Sept. 5 and when the fertility was maintained.

P62. How kind of grass pasture influenced persistency of milk production, yield of TDN and intake of dry matter when grazed by dairy cows. D. M. SEATH, C. A. LASSITER, JACKSON TAYLOR, and WILLIAM TEMPLETON, Kentucky Agr. Expt. Sta., Lexington.

Straight stands of Lincoln brome grass, orchard grass, Ky. bluegrass, and Ky. 31 fescue were established as experimental pastures in the fall of 1951. Each grass was assigned at random to two 2-acre plots, making a total of 8 plots. Besides the appropriate fertilization at seeding time, all plots received 100 lb. of nitrogen yearly, split into 3 equal applications during the growing season.

The persistency ratings for milk production (as a ratio between what cows produced during the 4th experimental wk. as compared to the wk. prior to the test period) were approximately the same for the years 1952 and 1953. The 2-year average was: for brome grass, 91.6%; orchard grass, 88.9%; bluegrass, 93.4%; and fescue, 72.7%.

Estimated yields of TDN per acre averaged for brome grass, 1,845 lb.; orchard grass, 1,613 lb.; bluegrass, 1,789 lb.; and fescue, 1,490 lb. The fescue yield was comparatively low for 1952 partially because of a thin initial stand, but for 1953 the yield was about the same as for the other grasses.

By using chromogen(s) and chromic oxide as indicators, estimations were made in 1953 as to intake of dry matter, and for 1952 and 1953 as to the apparent digestibility of dry matter and protein. The 1-yr. estimated average daily intake of dry matter per 1,000 lb. of body weight of cows was approximately the same for bluegrass, brome grass, and orchard grass, but averaged 3-4 lb. less for fescue. Only small differences were found between the grasses in digestibility of dry matter. For both years, however, orchard grass ranked highest (2-yr. av., 65.3%) and brome grass the lowest (2-yr. av., 60.8%). Bluegrass had a 2-yr. average of 62.0% and fescue 63.5%. Protein digestibility was highest for brome grass for both years and averaged 74.6%. It was lowest both years for bluegrass, with an average of 69.2%. The 2-yr. average for orchard grass was 73.9%, and for fescue, 72.2%.

P63. Dry matter intake and digestibility studies with grazing dairy animals — a progress report. W. A. HARDISON, R. W. ENGEL, G. C. GRAF, and W. N. LINKOUS, Virginia Agr. Expt. Sta., Blacksburg.

During the 1953 grazing season a combination of indicators — plant chromogen and chromic oxide — was employed to measure the consumption and digestibility of pasture herbage by lactating dairy cows. The cows were allowed to graze 3 different pasture mixtures. Half of the cows received grain; the other half no grain. From the 1st of June through the month of Sept. half of the pasture area was under irrigation; the other half received no supplemental water. Four digestion trials (4 cows in each), were conducted throughout the pasture season. Each trial consisted of a 7-d. preliminary period, during which time each cow received daily 15 g. of Cr_2O_3 in capsule form, and an 8- or 10-d. collection period. Grab fecal samples were obtained from each cow twice daily, at 6 A.M. and 4 P.M., and compounded during the collection periods. The average digestibility of the consumed D.M. ranged from 61-67%. The average digestible D.M. intake from grass varied from 19.0-24.6 lb/cow/day. It was observed that the cows consumed more of the irrigated herbage and that this herbage was about 5% more digestible than the nonirrigated grass. Although the feeding of grain had no noticeable effect on the digestibility of the consumed grass, it did decrease the pasture herbage intake about 25%. The effect of rotation grazing on the digestibility of herbage D.M. also was studied.

P64. The grazing behavior of lactating cows on pasture. H. L. FISHER, G. C. GRAF, W. A. HARDISON, and N. R. THOMPSON, Virginia Agr. Expt. Sta., Blacksburg.

The grazing behavior and related activities of 4 Holstein cows were observed for 2 periods

of 240 consecutive hr. during July and Sept., 1953. The cows grazed on 2 pasture mixtures, ladino clover–orchard grass and alfalfa–orchard grass. A system of rotational grazing was followed in which the cows were changed at intervals of 3 to 7 d. The first observation was made when the cows were on nonirrigated pastures and the second when they were on irrigated pastures.

The cows grazed an average of 9 hr., 2 min.; loafed 5 hr., 58 min.; and ruminated 8 hr., 38 min. daily. Fifteen hr. were spent in a standing position. The number of defecations, urinations, and drinks of water per day were 14.5, 9.5, and 4.0, respectively. Of the total grazing per 24 hr. period 51% was done at night.

P65. Pangola-white clover pastures for dairy cattle. S. P. MARSHALL, Univ. of Florida, Gainesville.

Two areas of pangola-clover pasture were grazed separately by dairy heifers and by lactating cows. These pastures were limed and received annual fertilizer applications totaling 90 to 126 lb. of P_2O_5, 180 lb. of K_2O and 143 to 193 lb. of N per acre.

Grazing rotationally on 2 pasture plots, heifers averaged 0.73 lb. gain daily during the 234-d. grazing season. They obtained an average of 6,753 lb. of TDN and gained an average of 625 lb. of body weight per acre of pasture grazed.

Lactating cows grazing pangola-clover pastures rotationally during 2 yr. obtained an average of 5,421 lb. of TDN annually per acre. They produced an average of 33.3 lb. of FCM daily and obtained an average of 60.2% of their TDN intake from pasture.

Good quality was maintained in the pasture into the fall, as indicated by the performance of the cattle and by the high moisture and crude protein contents of the forage. Pangola forage samples taken in June, July, Aug., Sept., and Oct. analyzed 15.6, 16.5, 17.3, 15.4, and 16.6% of dry matter, respectively, and 2.9, 2.9, 2.8, 2.8, and 3.1% of crude protein, respectively.

P66. Alfalfa–orchard grass, ladino clover–orchard grass, and ladino clover–Kentucky 31 fescue pastures for milk production. N. R. THOMPSON and C. W. HOLDAWAY, Virginia Agr. Expt. Sta., Blacksburg.

Pasture mixtures of alfalfa–orchard grass (A), ladino clover–orchard grass (B), and ladino clover–Kentucky 31 fescue (C) were grown on 2-acre lots in 4 replicates. Lots were grazed rotationally by lactating dairy cows, using a cross-over plan based on 3×3 Latin squares. Mean daily yields of FCM per cow were, for 1950: 32.5, 32.9, and 35.0 lb., for A, B, and C, respectively. Values for 1951 were (A) 36.1, (B) 40.0, (C) 34.6, and for 1952, (A) 27.2, (B) 27.4, (C) 23.7. A and B differed significantly for 1951 only. C differed significantly ($P < 0.05$) to highly significantly ($P < 0.01$) from B each year and from A the 1st and 3rd years. The superior stimulus of C on milk yield the first year was attributed to a preponderance of ladino clover in the mixture; the inferior effect of the 2nd and 3rd yr. to a decline of the clover and an increased growth of the fescue.

P67. Biochemical factors that affect the apparent nutritional value of lespedeza sericea. G. E. HAWKINS, JR., and K. M. AUTREY, Alabama Polytech. Inst., Auburn.

It has been assumed that the low nutritive value of sericea was due to its tannin content. In studies at this station, however, crystalline tannin mixed with alfalfa hay did not affect significantly the digestibility of dry matter or of crude protein. The present investigation was initiated to determine whether the leaf or the stem was the unpalatable part of sericea plants and to provide information on the composition and the digestibility of sericea leaves.

Twice daily during 5-d. test periods 5 dairy animals were fed more of the diets than they would consume. The test diets, all ground, were: AH, alfalfa hay; SH, sericea hay; SL, sericea leaves; ALSS, 52% alfalfa leaves and 48% sericea stems; and SLAS, 52% sericea leaves and 48% alfalfa stems. The mean daily consumption of the diets in lb. per 100 lb. of body weight, was: AH, 3.20; SH, 2.82; ALSS, 3.31; SLAS, 3.31; and SL, 2.88 and the L.S.D. ($P = 0.05$) was 0.36. The sericea leaves contained 18.43% protein and 5.22% ash on the dry basis and preliminary determinations indicate that the apparent digestibility of dry matter and of protein in sericea leaves was 43.89 and 35.96%, respectively.

P68. Type score as related to productive life, fat production and age at first calving. P. L. KELLY, H. P. DAVIS, and M. N. LAWRITSON, Univ. of Nebraska, Lincoln.

The over-all type, mammary gland score, and fat production records of 114 Holsteins were studied in relation to their productive life and age at first calving.

The type scores were obtained from photographs taken at first freshening. The fat production was adjusted to maturity by standard methods. When arranged in descending order of type scores by quartiles the type score avarages were: 83.0, 80.06, 78.11, 74.82; the quartiles for average productive life in days were: 1,332, 1,370, 940, and 915. The fat production averages by quartiles were: 524, 532, 468, and 464. When the data were arranged in descending order by quartiles for productive life, the quartiles averages in days for that feature were 2,152, 1,232, 786, and 410. The corresponding quartiles for the other factors were as follows: type score, 80.29, 78.74, 78.80, and 78.10; mammary score, 79.20, 76.30, 77.40,

and 76.05; and fat production, 541, 517, 485, and 446.

Correlations were as follows:

Productive life and mammary score = 0.209[1]
Productive life and type score = 0.215[1]
Productive life and fat production = 0.405[2]

[1] Significant — 0.05
[2] Significant — 0.01

P69. Genetic analysis of components of type and production in Ayrshires. A. E. FREEMAN[1] and R. S. DUNBAR, JR., West Virginia Univ., Morgantown.

All available daughter-dam comparisons on cows classified between July, 1950, and Sept., 1952, by the Ayrshire Breeders' Assoc. were used to estimate heritabilities of the type components and final type rating and the genetic correlation between each component and butterfat production. All estimates were based on an intraherd analysis of single type and butterfat records.

The estimates of heritability were as follows: head and neck, 0.30; shoulders and chest, 0.15; middle and loin, 0.31; rump and thighs, 0.32; feet and legs, 0.18; udder size and shape, 0.08; udder attachments, 0.06; teats, veins and quality, 0.27; general quality, 0.13; breed character, 0.32; and final type rating, 0.31. All components of type exhibited relatively high phenotypic and rather low genetic correlations with final type rating. The genetic correlations between the components of type and butterfat production ranged from –0.46 for head and neck and butterfat production to 0.55 for middle and loin and butterfat production; the phenotypic correlations between the components of type and butterfat production ranged from 0.00 to 0.14. The estimates of the phenotypic and genetic correlations between final type rating and butterfat production were 0.09 and –0.12, respectively.

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P70. The influence of dams and sires upon the breeding efficiency of their daughters within a Holstein-Friesian herd. M. A. INCHIOSA and K. O. PFAU, New Jersey Agr. Expt. Sta., Sussex.

These studies were undertaken in an attempt to investigate the extent to which female reproductive performance is inherited in dairy cattle. The data for these investigations were obtained from the records of the Overbrook Dairy Herd in Cedar Grove, N.J.

As a measure of the breeding efficiency of an animal, the average calving interval was used, this being the average number of days from one parturition to the next. A total of 338 dam-daughter comparisons were involved in comparing the breeding efficiencies of the daughters of 8 Holstein sires. Highly significant differences were found between the mean breeding efficiencies of the 8 groups of daughters.

The correlation between the breeding efficiencies of dams and daughters was calculated to be 0.203. The correlation between paternal half-sisters was found to be 0.176 and the correlation between individual reproductive cycle records of the same cow amounted to 0.135. All 3 were highly significant values.

Estimates of heritability were calculated and gave evidence that 41% of the differences observed between the mates of a sire in regard to their average breeding efficiencies were due to differences in their genetic constitutions.

P71. Physiological response and semen production of purebred and F_1 Red Sindhi crossbred Holstein-Friesian and Brown Swiss bulls exposed to extreme climatic conditions. H. NAELAPAA, J. E. JOHNSTON, and J. J. VIZINAT, Aluminum Co. of America and Louisiana State Univ., Baton Rouge.

Two bulls each of the Holstein-Friesian, Brown Swiss, and the respective Red Sindhi (F_1) crossbreds of these breeds were exposed for a period of 7 d. to controlled, cycled climatic conditions. The maximum daily temperature and humidity were 104° F. and 30 mm. Hg vapor pressure (54% relative), and the minimum 82° F. and 22 mm. Hg vapor pressure (79% relative), respectively. Body temperature, respiration rate, serotal length, and flank and serotal skin temperatures were determined twice daily after bulls had been exposed to maximum and minimum conditions. Two ejaculates of semen were collected weekly beginning 3 wk. prior to stress and ending 5 wk. after stress. Crossbred bulls showed lower body temperatures than the purebreds under both minimum and maximum conditions, but their respiration rates were lower only under minimum conditions and flank and serotal skin temperatures lower only under maximum conditions. These responses did not appear to be related to surface area per unit of body weight. Adverse effects on semen quality did not become apparent until 1 to 2 wk. after the end of stress. All purebred bulls showed marked reductions in initial motility and concentration of spermatozoa and increases in morphologically abnormal spermatozoa, whereas crossbreds showed only slight effects.

P72. Response of Holstein-Friesian, Jersey, Red Sindhi-Holstein (F_1), and Red Sindhi-Jersey (F_1) bulls to a simulated subtropical climate. H. KELLGREN, J. E. JOHNSTON, J. B. FRYE, JR., and B. GREEN, Lousiana State Univ., Baton Rouge.

In order to evaluate the effects of Louisiana summer climatic conditions on semen production and physiological responses of bulls, 2 Holstein-Friesian, 1 Jersey, 2 Red Sindhi-Holstein (F_1), and 2 Red Sindhi-Jersey (F_1) bulls were ex-

posed to cycled temperatures ranging from 75 to 95° F. at a humidity of 22 mm. Hg vapor pressure. One bull of each breed was maintained under natural (maximum temperature 75° F.) climatic conditions as a control. Two semen samples were collected from each bull each week commencing 8 wk. prior to initiation of stress and continued for 5 wk. after. Red Sindhi crossbreds maintained lower body temperatures and respiration rates than the purebreds under the elevated temperatures but were higher than any of the control bulls. Characteristics such as scrotal length and flank and scrotal skin temperatures did not reflect definite breed differences. All bulls exposed to elevated temperature showed decreases in initial motility and concentration of spermatozoa and increases in morphologically abnormal spermatozoa and methylene blue reduction time. Most pronounced effects were on purebred bulls. All bulls had returned to normal semen production by the end of the 5 wk. postexperimental period. Control bulls maintained semen quality throughout the experiment.

P73. Genetic variation and covariation in rate of maturity and level of production in dairy cattle. C. G. HICKMAN[1] and C. R. HENDERSON, Cornell Univ., Ithaca, N. Y.

For practical economic and biological reasons the use of progeny tests of bulls as a basis for selection has resulted in selection based largely on their daughters' first lactation records. The consequences of such selection on genetic improvement for lifetime production have been of concern to dairymen. Production records on artificially sired cows are of value for investigating this problem because variations due to sires, herds, and time trends can be separated. The artificial breeding program is too new to furnish adequate data on lifetime production, but the New York records on Holsteins include 3,912 artificially sired cows with both 1st and 2nd lactation records. By means of the variance components technique it was estimated that genetic variation of increase in production from 1st to 2nd lactation is only about 1/3 as great as genetic variation for level of production on first lactation. Also, there was some evidence of positive genetic correlation between 1st lactation production and increase from 1st to 2nd lactation. The results of this study do not confirm the frequently stated hypothesis that emphasis on first lactation records automatically selects bulls whose daughters do not produce well in subsequent lactations.

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P74. Genetic covariation between milk yield and fat percentage among herds stratified according to average fat percentage. B. R. FARTHING and J. E. LEGATES, North Carolina State College, Raleigh.

Many breeders of Holstein cattle are interested in raising the level of fat percentage in their herds. Guernsey and Jersey breeders are becoming interested in raising the level of milk production. Studies to date have indicated that milk yield and fat percentage are negatively correlated genetically. This fact would indicate that raising the level of production for one of these factors would be accompanied by a corresponding decrease in the other.

The present study was initated to determine the genetic correlation between milk production and fat percentage in herds with varying levels of fat percentage. Data studied consisted of 7,283 daughter-dam comparisons of Guernsey and Holstein sires used in Artificial Breeding Associations with D.H.I.A. proofs computed during 1949, 1950, and 1951. The study included 2,024 herds. The average test of all cows in each herd was used to stratify the data for each breed into 10 groups.

The genetic variance in fat percentage for Holsteins was approximately ½ that for Guernseys. The over-all genetic correlation between milk yield and fat percentage on an intrasire, intraherd basis was –0.56 for Guernseys and –0.42 for Holsteins. There was no distinct trend in genetic correlation or genetic covariance as the herd average for fat percentage increased from 4.4 to 5.3 in Guernseys. A slight increase in negative relationship was indicated in Holsteins as the herd average for fat percentage increased from 3.2 to 4.1. In neither Holsteins nor Guernseys was there a trend toward lower milk production as herd average for fat percentage increased.

P75. A comparison of approximate indexes for butterfat production. R. S. DUNBAR, JR.,[1] and C. R. HENDERSON, Cornell Univ., Ithaca, N. Y.

An intraherd selection index procedure requiring the solution to a single set of equations and which utilizes all records and simultaneously corrects for environmental trends has been published by Henderson. The ranking of 57 cows indexed by application of this procedure to 452 records on 142 cows accumulated during an 18-yr. period has served as a basis for comparing certain "approximate" indexing procedures. Rank correlation coefficients were utilized to measure the degree of similarity between the rankings obtained by several "approximate" indexes and the "best" index. Approximate indexes considered included five which required solving equations with varying subsets of data and, in certain instances, with records expressed as deviations from respective annual averages. Other approximate indexes examined, which required particular tabulations of the data, were the index described by Legates and Lush, a modification of their index, estimated real pro-

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ducing abilities, the individuals' production average, and a ranking made by the herd owner.

These comparisons indicate that there is an advantage in combining the available information such that only a single set of equations need be solved, and also that, in the absence of computing facilities for such a procedure, certain approximate indexes are accurate enough for practical purposes.

P76. Histological development in the postnatal bovine testis. R. G. FOSSLAND, Univ. of Nebraska, Lincoln.

Unilateral orchectomies were performed at fortnightly intervals on 59 bulls ranging in age from birth to 18 mo. Sections were made with the paraffin method from 3 loci, namely *(a)* the caudal epididymis and adjacent testis, *(b)* the caput epididymis and adjacent testis, and *(c)* a deep transverse section of the mediastinum.

Histological features noted were: *(a)* There is a 4-fold increase in diameter of the seminiferous tubules between the 1st and 18th mo. *(b)* The mediastinum and rete occupy a deep central location along the longitudinal axis of the testis. *(c)* Because there is a cyclical disposition of the cytological elements in a longitudinal section of the tubules, development of which proceeds in a wavelike manner, it is necessary to observe either a large area or many serial sections. *(d)* A peculiar canalization of the mucosa layer of the ductus deferens was noted in a number of individuals. *(e)* Spermatozoa in the lumina of the seminiferous tubules and epididymi were not noted until the 9th mo. postpartum in Jerseys and 10th mo. in Holsteins. *(f)* Dramatic regressive changes were noted in the 2nd testis from individuals who suffered complications following the 1st orchectomy.

P77. Beta-hydroxybutyric acid metabolism of the mammary gland.[1] SOMA KUMAR and S. LAKSHMANAN, Univ. of Maryland, College Park.

Beta-hydroxybutyric acid has been recognized as one of the major metabolites of the lactating mammary gland of ruminants from the extent of its absorption from the blood by the gland. In order to determine the fate of beta-hydroxybutyric acid in the gland a study was made in which an isolated mammary gland of a lactating cow was perfused with blood containing 1 mc. of sodium beta hydroxybutyrate-1-C^{14}. The higest C^{14} activity appeared in the milk fat. The milk fatty acids were fractionated and the specified activities of individual acids on a molar basis were determined. All the steam volatile acids were found to be labeled; the activity increased from butyric to caprylic acids and declined in capric acid. Lauric acid had very little activity. The results indicate that, besides acetate, beta-hydroxybutyrate must be regarded as a major precursor of short chain fatty acids of ruminant milk fat.

[1] Work done under Contract AT (30-1)-881 of the U.S. Atomic Energy Commission.

P78. Recent studies of the antagonism between mammary growth and lactation. JOSEPH MEITES and J. T. SGOURIS, Michigan State College, East Lansing.

It was recently demonstrated that the ability of a moderate dose of prolactin to initiate lactation in rabbits with well developed mammary glands could be effectively inhibited if optimal, mammary growth-promoting levels of estrone and progesterone were injected at the same time. In the present rabbit experiments it was found that even in the presence of optimal amounts of the 2 steroid hormones, large doses of prolactin could elicit at least a fair degree of lactation. If less than optimal amounts of the 2 steroids were injected, then moderate doses of prolactin were also effective in initiating lactation in the rabbits. These findings suggest that *(a)* lactation is not initiated during pregnancy because the level of prolactin secretion is too low to overcome the inhibitory effects of the 2 ovarian hormones on the mammary gland and *(b)* lactation can continue in animals which subsequently become pregnant because the milking act maintains prolactin secretion at a sufficiently high level to overcome the mammary inhibitory effects of the 2 ovarian hormones.

P79. Stimulation of growth and function of the rat mammary gland.[1] W. R. KIRKHAM[2] and C. W. TURNER, Univ. of Missouri, Columbia.

In a continuation of previous experiments on normal rats nucleic acids were used to study the extent of mammary growth and function that could be induced by hormone therapy. Castrate female rats received injections of progesterone and estradiol-benzoate (5 mg: 1γ) daily for 15, 21, 25, and 32 d., and the glands were examined for nucleic acid content. At 15 d. the total desoxyribonucleic acid (DNA) was as high in the treated animals as in normal pregnant controls. At the 21, 25, and 32-d periods the gland growth (DNA) was equivalent to that of normally lactating rats but not particularly higher. In terms of functional capacity (ribonucleic acid, RNA), all of these glands were comparable to normal glands of 12 to 15 d. of pregnancy and did not show the increase in RNA shown in normal glands of late pregnancy and lactation. Histologically these glands showed good lobule-alveolar formation. The lobules did not show the expanded condition seen in lactating glands. Cellular growth comparable to the lactating condition seemed to be established.

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[2] Postdoctoral fellow of the National Cancer Institute.

An attempt was made to induce lactation in castrate female rats receiving progesterone and estrogen (5 mg: 1γ, 21 d.) by administration of cortisone and lactogenic hormone. The glands showed no difference to the above in terms of nucleic acids but histologically they appeared to be comparable to glands of the normal 2-d. lactating female. Further studies on glands grown by progesterone-estrogen administration have demonstrated that by varying the relationships between progesterone, estrogen, and a crude lactogenic hormone preparation, these castrate females can be brought into good lactation.

P80. The effect of daily epinephrine injections on lactation in the rat as indicated by growth of the young. N. L. VanDemark and R. L. Hays, Univ. of Illinois, Urbana.

Previous investigations have shown that daily injections of epinephrine reduce mitotic activity in epithelial tissues. No known investigations have been made involving the secretory epithelium of the mammary gland though the short term effects of epinephrine on milk ejection have long been known. In order to test the longer term effects of epinephrine on mammary function, daily injections of epinephrine (0.2 ml. 1:1,000 sol. adrenalin-hydrochloride) were given to female rats starting on the day after parturition and continued until the young were 28 d. old. The litters were standardized to 7 young and daily weights were recorded for both the mothers and young. No appreciable wt. changes in the mothers were observed. By the 5th d. of age the young of 10 treated females were 10% lighter than were the young of 10 controls. This difference increased to 20% at 10 d. and to 25% at 15 d. In other females daily epinephrine injections during gestation and lactation resulted in an even slower growth rate of the young. The slower growth rate is believed to be due to a reduced production of milk resulting from the action of epinephrine on the secretory epithelium of the mammary gland.

P81. Cause of the differences in the productivity of dairy cattle.[1] C. W. Turner, Univ. of Missouri, Columbia.

The endocrine research of the past 25 yr. has indicated the following causes for differences in productivity ability of dairy cattle: *(a)* The extent of udder development is under endocrine controls. *(b)* The initiation and maintenance of lactation is dependent upon the secretion of the lactogenic hormone. *(c)* Energy metabolism is controlled by the pituitary and thyroid gland. *(d)* The mobilization of the milk precursors is controlled by the growth hormone, adrenal and pancreas. *(e)* Calcium and phosphorus are controlled by the parathyroid hormone. *(f)* Harvesting of milk is controlled by the milk "let-down" hormone.

When one or more of the endocrine glands secrete limited amounts of their important hormones, milk production will be limited proportionally. Low-producing cows are now discarded, but it is not known whether their poor production is due to one endocrine deficiency or many. Some cows will respond greatly when one hormone deficiency is corrected; failure of others to respond is due, it is believed, to other milk limiting hormone secretion rates. It is necessary to develop direct and indirect methods by which the endocrine gland secretion rate may be measured in calves and milking cows. It is also necessary to determine the mode of inheritance of endocrine gland secretion rate in cattle.

P82. Specific activity-time relationship in tracer studies of milk secretion.[1] S. Lakshmanan and Soma Kumar, Univ. of Maryland, College Park.

The major problem in specific activity-time studies of milk secretion is the impossibility of sampling milk representative of the mammary gland pool at any given time. One can only collect milk formed over a period of time and obtain the weighted averages of the continuously varying specific activities of the milk components secreted during that interval. Factors involved in the experimental ejection of milk impose a practical limitation on the number of times an animal can be milked in any specified period. Therefore, the effect due to the storage of milk in the udder is usually substantial, and because of this the specific activity of a particular precursor in blood and its product in milk will not exhibit the relationship predicted by the theory of Zilversmit *et al. (J. Gen. Physiol.* **26**: 325. 1943), even if the basic assumptions of the theory apply. Once this is realized, it is possible to interpret data of specific activity-time studies in experimental alterations of the rate of milk secretion in intact animals.

P83. ATP-ase activity in lactating bovine mammary particulate. J. E. Gander and W. E. Petersen, Univ. of Minnesota, St. Paul.

Studies on the ability of bovine mammary mitochondria to cleave ATP (adenosine triphosphate) and UTP (uridine triphosphate) were made. Fractionations were carried out by ion exchange methods of Cohn and Carter. It was noted that freshly prepared mitochondria would bring about an uptake of inorganic phosphate in the presence of ATP, Mg, and K, but this was not observed in the presence of UTP, Mg, and K. The phosphorylitic cleavage of these 2 compounds seemed to go at about the same

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rate, although no attempt was made to determine if the same phosphatase was responsible.

Myokinase activity was also shown to be present by an increase in AMP during the experimental period. With ion exchange methods there was some evidence that other organic phosphate compounds were being formed besides ADP and AMP. Similar studies were carried out on the whole homogenate and supernatant from the mitochondrial fraction.

P84. Oxidative metabolism in lactating bovine mammary particulate. R. S. ADAMS and J. E. GANDER, Univ. of Minnesota, St. Paul.

Oxidative metabolism of mitochondrial preparations from lactating bovine mammary tissue was studied. The rate of oxidation of such intermediates as D-ketoglutarate, citrate and succinate was shown to take place. The rate of succinate oxidation was 5- to 10-fold that of citrate. This seemed to follow the observations of Moore and Nelson, who observed poor citrate oxidation in guinea pig mammary mitochondria. The possible function of Ca ions on citrate inhibition was studied with oxalate and ethylene diamine tetra acetate. Also, the rate of oxidation of citrate in rat kidney mitochondria with or without added mammary mitochondrial dialysate was studied.

The influence of H ion concentration (pH 6.6 to 7.4) on citrate oxidation was studied. Observations upon oxalacetate, acetic and acetic-oxalacetate oxidation were made. Succinate oxidation was further studied by determining the activity of the succinoxidase system and the cytochrome oxidase system independently.

P85. The effect of estrogen and progesterone on the distribution of alkaline phosphatase and glycogen in the bovine uterus. T. R. WRENN, S. MOSS, and J. F. SYKES, Dairy Husb. Research Branch, USDA, Beltsville, Md.

Three mature castrated cows were used for these studies. Uterine tissue was obtained for study by biopsy. After castration, phosphatase disappeared from the surface epithelium and from the dense upper endometrial stroma. The amount of phosphatase in other structures normally containing phosphatase was decreased. Glycogen increased in the surface epithelium and to some extent also in the upper endometrium.

When these cows were brought into heat with estradiol benzoate (1.8 mg. total dose), phosphatase returned to the surface epithelium and increased in the loose areolar tissue in glandular areas but still was absent from the dense upper endometrial stroma. Glycogen was absent from the surface epithelium.

After a period of rest, during which time the distribution of phosphatase and glycogen returned to the castrate levels, either 20, 40, or 60 mg. of progesterone per day was given for a period of 5 days. Progesterone produced a heavy concentration of phosphatase in the distal border of the surface epithelium. There was a marked increase in the phosphatase content of the connective tissue stroma and, at the highest dose of progesterone employed, phosphatase was present in the dense upper endometrial stroma. Very heavy deposits of glycogen occurred in the surface epithelium.

While differing in some details, estrogen administration produced a pattern of phosphatase and glycogen distribution most characteristic of that seen during mid cycle (days 8-16) in the uterus of cycling cows. Progesterone, however, produced a pattern similar to that seen in the early and late stages of the heat cycle.

P86. The agglutination test in the study of the microflora of the bovine vulvo-vaginal mucosa. J. E. VERNA, B. WASSERMAN, and B. W. HENDERSON, JR., Univ. of Rhode Island, Kingston.

A total of 62 samples of vulvo-vaginal mucosa were secured from animals of varying ages. Most of the samples were taken by curette scrapings, but some biopsies also were obtained. Care was used at the time of sampling to avoid all extraneous material. All samples were inoculated onto duplicate sets of blood and tryptose agar plates and incubated for 24 hr. at 37° C. One set of plates was incubated aerobically and the other anaerobically.

Approximately 50 different organisms have been isolated. Using cellular suspensions of these organisms as the antigen, agglutination against sera of cows of varying ages was observed. The amount of diluted sera used in the test was 0.5 ml. and the quantity of antigen suspension was 0.1 ml. The titers were read after 2 hr. at 37° C. and again after overnight incubation at 55° C. Preliminary results indicate better agglutination and sharper demarcation of the titler between age groups when heat-inactivated sera are used.

Titers of 640 were obtained with certain Gram-positive cocci and sera from older cows. Calf sera usually gave lower titers. Organisms that react with highly diluted sera will be identified and their frequency of occurrence determined.

Preliminary indications are that some organisms found on the vulvo-vaginal mucosa have at some time invaded the tissue, as shown by the concentration of specific agglutinins present in the blood of the cows.

P87. Undecorticated safflower meal and protein content of grain for milk production. R. H. ROSS, G. W. CLEVELAND, and D. L. FOURT, Univ. of Idaho, Moscow.

A double change-over feeding trial with 18 Holstein cows was conducted to compare 3 grain rations for milk production when fed with good quality chopped alfalfa hay fed free choice. The first trial consisted of three 4-wk. periods

with 1-wk. adjustment periods between each. Grain was fed at the rate of 1 lb. for each 5 lb. of milk and was adjusted weekly. Ration A consisted of 1/3 barley, 1/3 oats, 1/6 dried molasses beet pulp, and 1/6 wheat bran; Ration B contained 20% unhulled safflower meal; and Ration C, 18% linseed oil meal. Crude protein contents were 11.6, 12.9, and 17.4% for Rations A, B, and C, respectively. Iodized salt and bone meal were added to the grain at the rate of 1% each. Grain and hay consumption were the same for all groups. Daily milk production (FCM) was 41.2, 39.8, and 40.4 lb., and the total feed cost per 100 lb. FCM was $1.94, $2.04, and $2.12 for rations A, B, and C, respectively. This feeding trial is being repeated with 5-wk. periods.

P88. Effect of certain factors on the diurnal excretion pattern of chromic oxide. J. C. MAHAFFEY, W. J. MILLER, J. D. DONKER, and H. L. DALTON, Univ. of Georgia, Athens.

Four Jersey steers were used in chromic oxide balance trials. Total fecal collections were made at 2-hr. intervals for the last 3 of 7 days in which feed intake was stabilized and 10 g. of chromic oxide administered daily. Each steer received 4 roughages, 4 feeding schedules, and chromic oxide in 4 forms. On rations of alfalfa hay, crimson clover silage, or a mixture of these the lowest av. concentration of fecal chromic oxide was 0.28% at 6 A.M. and the highest 0.37% at 6 P.M. The daily ration was fed in 1, 2, 3, or 6 portions and 4 hr. allowed for the consumption of each portion. The greatest range of chromic oxide concentration in feces occurred when steers were fed 6 times daily and the smallest when fed once daily. When chromic oxide was given in pure form or dried with collodion the range of fecal concentration was smaller than when it was given as a gelatin suspension or as a baked-flour paste mixed with grain.

P89. An improved method of using plant pigments as an indicator of digestibility. E. A. KANE and W. C. JACOBSON, Dairy Husb. Research Branch, USDA, Beltsville, Md.

It has been shown by Irvin *et al.* that pheophytin, a decomposition product of chlorophyll, is the most significant plant pigment in cow feces. Apparently most of the chlorophyll, in passing through the bovine intestinal tract, is converted to pheophytin. Since pheophytin displays its maximum optical density reading at 415 mμ, all spectrophotometric readings were made at 415 mμ.

After some experimentation it was found that if plant pigment extracts from freshly-cut forages are treated with saturated oxalic acid, the maximum optical density reading is changed from 435 mμ (chlorophyll) to 415 mμ (pheophytin). Hence a method is available to duplicate the changes chlorophyll undergoes in the cow's digestive tract.

If pheophytin is defined, not as a definite chemical compound but as the optical density readings of oxalated plant pigments read at 415 mμ, then it is possible to determine digestibility by pheopytin ratios in feed feces.

Experiments have been conducted on orchard grass pasture clippings and on mixed clover grass pasture clippings in order to compare the digestibility values obtained by pheophytin with dry matter digestibilities obtained by the standard total collection procedure. Results in both experiments indicate satisfactory agreement. Theoretically, the pheophytin method should have greater accuracy over the other plant pigment methods, since one pigment predominately determines the readings.

P90. A survey of the condition of cows at the time of artificial breeding. F. H. FLERCHINGER and R. E. ERB, State College of Washington, Pullman.

This survey was conducted in cooperation with 8 experienced technicians employed by Northwest Coop. Breeders, Mt. Vernon, Wash., from April through Oct., 1952.

Each technician made observations on the condition of each cow at the time of breeding considering: *(a)* the appearance of the mucus, *(b)* the condition of the cervix, *(c)* the condition of the uterus, and *(d)* whether the inseminating tube was passed through the cervix. A total of 11,956 observations were made, of which 17.8% were made on heifers.

No mucus was recorded for 53.7% of the services; 41.6% was clear; 2.8%, slightly cloudy; 1.0%, cloudy; 0.7%, purulent; and 0.2%, bloody. A normal cervix was noted for 84.5% of the services with 14.7% enlarged, and 0.8% small. Normal uteri were observed at 88% of the services with 10.2% enlarged. 0.3% small, and 1.5% not observed. The inseminating tube was not passed through in 39.5% of the heifers and 4.4% of the cows.

Normal services were regarded as those with clear mucus, normal cervix, normal uterus, and the inseminating tube passed through. With these criteria 67.0, 69.9, 73.0, and 75.4%, respectively, of 1st, 2nd, 3rd, and 4th services were abnormal. Percentage abnormals were 24.6, 48.4, 33.1, 44.6, 25.0, and 24.4, respectively, for cows with clear, slightly cloudy, cloudy, purulent, bloody, and no observed mucus.

Cows with normal cervixes showed 62.8% abnormal in other respects compared with 88.3 and 89.6%, respectively, for enlarged and small cervixes.

Uteri classifications of normal, enlarged, small, and no observation showed, respectively, 64.3, 97.2, 93.8, and 89.2% abnormal in other respects.

P91. Calving age and intervals between calvings for successive calvings, first through tenth. H. P. DAVIS and BENJAMIN BROST, Univ. of Nebraska, Lincoln.

This study is based upon the records of the University Holstein herd for the period 1897-1948, inclusive, and represents 967 calvings, of which 884 followed a normal gestation. The average abortion rate was 8.56%, varying from 5.69% at the 1st calving to 13.73% at the 6th. The average age in days for the normal calvings at each successive calving was as follows: 1st, 899.6; 2nd, 1,359.4; 3rd, 1,819.3; 4th, 2,248.5; 5th, 2,717.6; 6th, 3,124.2; 7th, 3,526.8; 8th, 4,319.0; 9th, 4,824.3; and 10th, 4,903.4. The last figure was affected by one abnormally long interval out of 9 calvings. When the abortions were included, the age for all calvings in days was shortened as follows: 1st, 1.1; 2nd, 13.7; 3rd, 10.6; 4th, 8.2; 5th, 14.1; 6th, 23.8; 7th, 21.5; 8th, 390.8; 9th, 409.3; and 10th, 42.4.

The average calving intervals in days, for the normal calvings, were as follows: 1st-2nd, 456.1; 2nd-3rd, 460.4; 3rd-4th, 425.3; 4th-5th, 456.8; 5th-6th, 429.5; 6th-7th, 450.7; 7th-8th, 465.5; 8th-9th, 452.0; 9th-10th, 511.0, with an average of 451.1 d. (14.79 mo., or 1.24 yr.).

When compared with all calvings, including abortions, the average calving interval was 10.5 d. longer for the normal group. There was no distinct influence of successive calvings upon the length of the calving interval.

P92. Relationship between number of services and per cent nonreturns, calving rates and sex ratio in artificial breeding. R. H. FOOTE and ALETHEA C. HALL, Cornell Univ., Ithaca, N. Y.

Much research in artificial breeding and reproduction has of necessity relied primarily on nonreturn rates as the measurement of fertility. In order to have a standard of comparison with other measurements of fertility, 2,300 member herds of an artificial breeding organization cooperated in providing calving and other breeding information on nearly 50,000 cows. The 150-180 d. per cent nonreturns for cows inseminated 1, 2, 3, 4, 5, 6, and 7 or more times were 68.2, 64.6, 70.1, 66.3, 63.5, 63.4, and 54.4, respectively. Corresponding conception rates were 65.6, 59.4, 54.2, 47.3, 33.9, 33.3, and 21.0%, and actual calving rates (conception rate minus pregnant cows sold before calving) 59.3, 52.7, 47.6, 41.7, 30.0, 31.2, and 19.1%, respectively. The 150-180 d. per cent nonreturns for all cows (204,513) inseminated by this organization during the same period of time were 68.8, 64.8, 73.4, 66.3, 64.4, 60.9, and 61.5. The percentage of males in single births was 50.8, 51.1, 51.0, 50.3, 50.3, 30.2, and 26.7. Multiple births occurred in 2.6% of the cases, and 49.3% of these calves were males.

P93. Breeding efficiency as related to number of gestations of cows. M. H. EHLERS, R. A. MORRISON, and R. E. ERB, State College of Washington, Pullman.

A breeding efficiency study of records in a Holstein herd over a 25 yr. period included 2,176 cows having from 1 to 16 consecutive gestations. A total of 7,498 gestations and 19,714 services were represented. The average number of services per conception for 2,119 1st gestations of all cows was 2.76; for 1,371 2nd gestations, 2.62; 1,012 3rd, 2.26; 805 4th, 2.31; 628 5th, 2.57; 491 6th, 2.54; 360 7th, 2.73; 263 8th, 3.02; 188 9th, 3.19; 117 10th, 3.02; 144 > 10th, 3.74; 7,498 total, 2.63.

In the 771 gestations of cows with a single gestation, an average of 2.99 services per conception was required. With a total of 714 records made up of 2 consecutive gestations, number of services averaged 2.79; 614 of 3, 2.64; 684 of 4, 2.50; 712 of 5, 2.82; 763 of 6, 2.45; 663 of 7, 2.59; 618 of 8, 2.40; 655 of 9, 2.73; 418 of 10, 2.57; 888 > 10, 2.16.

The number of days from previous calving to present conception was lowest preceding the 3rd and 4th gestations. Percentage of cows conceiving on first service was highest with 4th gestations.

P94. The effect of the length of intervals between milking on the milk and butterfat production. J. H. KOSHI and W. E. PETERSEN, Univ. of Minnesota, St. Paul.

Ten complete lactations of 7 sets of identical cattle twins were used in this study in which 1 member of each set was milked at 12 - 12 hr. intervals and the other at 10 - 14 hr. intervals throughout the entire lactation. The butterfat content was determined weekly by the Babcock method on the A.M. and P.M. milk separately. Daily milk weights also were kept.

In 10 complete lactations the animals milked at 12 - 12 hr. intervals produced on the average 5,542.7 lb. of milk testing 3.80% and 210.5 lb. butterfat. Animals milked at 10 - 14 hr. intervals produced 5,647.7 lb. of milk testing 3.78% and 213.7 lb. of butterfat. Thus, animals milked at 10 - 14 hr. intervals produced 101.89% as much milk and 101.52% as much butterfat as those milked at 12 - 12 hr. intervals. The production for the 10-hr. interval, which is equal to 41.7% of the day, was 44.2% of the milk and for the 14-hr. period, which is equal to 58.3% of the day, was 55.8% of the milk. The milk secretion rate was 10.7% greater for the 10-hr. interval than the 14-hr. interval. The highest production in this trial was 9,645.0 lb. of milk and 324.2 lb. of butterfat.

P95. Response of the milking cow to various methods of management immediately following parturition. W. M. DUPRE and W. A. KING, South Carolina Agr. Expt. Sta., Clemson.

Three hundred sixty cows were divided into 5 equal groups to study the effect of different methods of management employed during the 1st 4 days after parturition. The methods included: Group 1 — hand milked in box stall, calf remaining with the cow; Group 2 — machine milked in box stall, calf remaining with

cow; Group 3 — hand milked in box stall, calf removed from cow; Group 4 — machine milked in box stall, calf removed from cow; and Group 5 — machine milked in milking parlor, calf removed from cow. The average daily milk production for the 26-d. period from the 5th through the 30th d. postpartum inclusively was: Group 1, 43.6 lb.; Group 2, 41.6 lb; Group 3, 44.0 lb.; Group 4, 44.2 lb.; and Group 5, 43.5 lb.

The data were analyzed by analysis of variance. The difference in milk production between hand-milked and machine-milked cows was not significant. The effect of the calf remaining with the cow was noticed not only during the 1st 4 d. but also for 3 d. thereafter. The cows with which the calves remained for 4 d. produced from 2 to 11 lb. less milk on the 5th day than did those from which the calf was removed.

P96. The effect of wind on Holstein, Brown Swiss, Jersey, and Brahman cows.[1, 2] SAMUEL BRODY, Univ. of Missouri, Columbia.

Cows were exposed for 2 weeks to each wind, 0.4, 5, and 9 mph, at temperatures 17°, 50°, 65°, and 80° F. and 65% humidity. Increasing wind from 0.4 to 9 mph at a given temperature showed no appreciable effects on milk production, feed and water consumption, and body weight. However, the heat production at 17° F. was 20 to 30% higher at the 9 than 0.5 mph wind for the Holstein, Swiss, and Jersey, and 60% higher for the dry Brahman. The difference in effect of wind on feed consumption and heat production may have resulted from having the fans turned off during feeding, milking, and cleaning but on during the measurements of heat production. Increasing wind from 0.4 to 9 mph did not affect heat production at 50°, 65°, and 80° F.; it increased the convective and reduced the evaporative heat loss; it reduced the rates of respiration, pulmonary ventilation, and respiratory vaporization. The animals were also exposed for 3 days to 95° F. when increasing wind increased the feed consumption and milk production and reduced the skin and rectal temperatures of the Holstein and Swiss cows.

[1] Abstract of three MSS for Mo. Research Bulls. in the Environmental Physiology and Shelter Engineering Series of the Mo. Agr. Expt. Sta. Authors include: S. Brody, H. H. Kibler, A. C. Ragsdale, H. J. Thompson, D. M. Worstell, and R. G. Yeck.

[2] Journal Series Paper No. 1414 approved by the Director of the Missouri Agricultural Experiment Station. "Thyroid Activity data at various temperatures were obtained with the aid of the U.S. Atomic Energy Commission."

P97. The role of the testis in mammary gland duct growth. R. P. REECE, New Jersey Agr. Expt. Sta., New Brunswick.

An experiment was carried out to determine whether mammary gland growth could occur in the noninjected rat in the absence of the testis. Thirty 21-d. old rats were castrated and fed a restricted diet for 10 d. Fifteen rats were continued on the restricted diet for an additional 4 d. and the remaining 15 rats were fed ad libitum for an additional 4 d. On the following day the rats were killed, and a mammary gland was removed from each rat and examined for evidence of growth. None of the glands from the control rats were positive, whereas 13 of the 15 glands from rats fed ad libitum for the final 4 d. were positive.

The effect of testicular stimulation on mammary growth was determined in thirty 21-d. old male rats fed a restricted diet for 14 d. Fifteen rats were injected subcutaneously daily with 25 rat units of PMS for the last 12 d. The rats were then killed, and a gland was removed from each rat and examined for evidence of growth. None of the glands from the noninjected rats showed evidence of gland growth, whereas 14 of 15 glands of the injected rats were positive.

EXTENSION SECTION

E1. Dairyman's "Bridge of Life." W. A. DODGE, Univ. of Vermont, Burlington.

This bridge of life was developed by the extension dairyman and a committee of county agents to draft a long-time dairy program. It presents the business of dairying from wedding bells to retirement.

Supporting this bridge are 3 abutments: breeding for production, a sound replacement program, and feeding and managing for production.

Other factors affecting the trip of the average dairy family across the bridge of life are soil, roughage program, farm layout, buildings and machinery, herd health, capital investment, farm, family interests such as 4-H club work, markets, and price levels.

The main purpose of this structure is to assist farm families in getting across the bridge without meeting financial disaster. All county agents have been provided with a replica of this painting. They have used it as an introduction to various types of meetings which point out the magnitude of the over-all program. This picture is helpful in individual farm planning. It makes an excellent analytical device to bring to the front what otherwise might seem to be a rather obscure weakness in individual farm plans.

E2. Use of television in dairy extension programs. D. L. MURRAY, Michigan State College, East Lansing.

Television provides an excellent medium for disseminating educational information by means of demonstrations and visual aids in presenting subject matter. This teaching technique has been used for years by extension workers in group meetings and has proved to be most effective. Field activities are important from the

standpoint of keeping in contact with problems of the industry and views of the dairymen. However, competition for the dairymen's time in attending meetings makes it necessary to evaluate field activities to determine how some of this time can be more effectively utilized by means of television.

The general reaction by extension workers to accepting television as a method of teaching is that it requires too much time to plan the show, prepare the visual aids, rehearse, and televise the program. Much time can be saved if visual aids for extension meetings are prepared in such a way that they may be used for a television program also. As experience is gained in putting on these shows, less time will be required for rehearsing.

The fact that the viewing audience is not large at this time may be an advantage, since experience gained now may make it possible to do a better job in the future.

E3. New developments in dairy visuals. G. F. JOHNSON, The Pennsylvania State Univ., State College.

This presentation is a demonstration of new developments in methods and equipment for doing visual teaching in dairy extension. The demonstration is divided into 3 sections: *(a)* visual aids and equipment for lantern slide lecture work; *(b)* motion picture production, and *(c)* visual aids for television.

The advantage of high wattage projectors, square screens, well-selected slides, and smooth presentation will be featured in the discussion of lantern slides. The making of tabulations and charts of maximum readability when projected as slides will be demonstrated.

The magnetic sound technique for producing inexpensive sound motion pictures will be demonstrated. Film will be changed from a silent to a sound film while the audience observes the procedure.

The importance of having pictures horizontal rather than vertical, having dull rather than glossy finish, and making proper selections of slides and movies for television will be discussed.

The importance of a drastic reappraisal of visual aids, a comprehensive "retooling" of extension agencies for visual teaching, and a progressive training program to bring all workers up to date will be emphasized.

E4. National survey of production testing programs. WM. PATTERSON, Virginia Polytech. Inst., Blacksburg, and J. E. STALLARD, Univ. of Wisconsin, Madison.

Replies were received from 45 state extension dairymen. Average herd size varies from 10.8 (Ala.) to 85.3 (Fla.) cows. Thirty-one states have only old type associations (av. 490 cows per supervisor) and 14 states have both old type and central laboratories (av. 1,350 cows per supervisor). Twenty per cent of cows reported on test are owner sampler. Rates vary from $6.50 to $13.50, 25 cows standard; $1.50 to $3.50, 10-cow owner sampler; 10 to 25¢ per cow over 10.

Five states use forms other than BDI. Three states use calendar month only, 6 others partly. Ten states keep feed records only on concentrates; 14, no feed records on most herds; some with roughage on herd basis only.

Training schools vary from on-job only to 3 wk. Many hold district work shops. Supervisor salaries are from $116 to $520 — from all of income to monthly salary to percentage of income; technicians (lab.) from $100 to $225 or on hourly basis. From 3 to 100% of herd books are checked annually; most states, 5 to 10%.

AR and HIR testing vary from state office supervisors only to nearly all association supervisors. Nine states have DHIA federations, 11 others organizing. Use of IBM is increasing, at least one state (Utah) computing all barn sheets.

E5. Survey on weighing and sampling milk for production records when pipeline milkers are used. M. E. SENGER, North Carolina State College, Raleigh.

E6. New York production and feeding analysis for DHIA herds. J. D. BURKE, Cornell Univ., Ithaca, N. Y.

A method of analyzing the feeding program of each DHIA herd and correlating the amount and quality of feed fed with production results will be presented. Special DHIA forms for this procedure will be presented with an explanation of their use.

The production and feeding analysis is a part of the annual DHIA herd summary. Copies are returned to the herd owner for his information and to the county agricultural agent for use in educational programs. The yearly production, amounts and quality of feed, costs, and value of product are punched on I.B.M. cards for group study and analysis.

E7. Source of nutrients and their effect on production in DHIA herds. C. M. CHANCE, Cornell Univ., Ithaca, N. Y.

DHIA production and feeding summaries reported in 1 calendar yr. from 2,450 Holstein herds were used in the study. The data were first sorted into various butterfat production groups with a 50 lb. interval between groups. The data were then analyzed within each fat group to study the effect of each of the following on milk and fat production: lb. concentrate fed; lb. and quality of hay fed; lb. and quality of silage fed; days and quality of pasture; cow size; herd size.

The average New York Holstein DHIA

herd produced 10,700 lb. milk and 384 lb. butterfat. These herds averaged 30 cow-yr. and 1,210 lb. body weight. They received on the average 3,130 lb. concentrate, 7,600 lb. silage, 4,300 lb. hay, and 160 days pasture. As milk and fat production increased, there was also an increase in body size and concentrate and silage consumption. Hay consumption, days on pasture, and herd size remained approximately the same for all fat groups.

TDN fed and required was determined in each of the studies. In most cases there was a surplus of nutrients fed. There was more variability within fat groups than between fat groups in this respect. When the data were considered disregarding fat groups, the surplus of nutrients over requirements was greater in the higher butterfat production groups. The higher butterfat production herds fed considerably more than 3,130 lb. concentrate and better than average roughages and pasture.

E8. Presentation and discussion on changes of DHIA forms. J. F. KENDRICK, USDA, Washington, D. C.

E9. How we encourage 4-H Club members to keep dairy production records in Kansas. E. R. BONEWITZ, Kansas State College, Manhattan.

The object of the 4-H Club dairy production contest, besides developing boys and girls, is to increase the interest in dairy club work and to stimulate the more universal adoption of practical and economical methods dealing with the feeding and management of producing dairy animals.

Monthly reports on cow's production and feed costs are mailed by 4-H Club members to county extension agents, who in turn send in material to the state dairy extension office. The 4-H boys and girls whose cows produce the most butterfat for the month are listed in the state Dairy Herd Improvement monthly newsletter. Winners in state contest are selected by means of a score card which covers feeding of the cow, milk and butterfat record, completeness and promptness of records submitted, story on "How I cared for my cow," and the 4-H club record. Winners in state contest in each breed are sent to the National Dairy Congress at Waterloo, Iowa.

Agricultural agents and club agents use a program in the county similar to the state program. Agents encourage civic clubs to help sponsor the dairy records program. Dairy Herd Improvement supervisors and artificial breeding inseminators give much support to the program. 4-H Club leaders are trained at state leaders' conference on the 4-H dairy production program.

E10. Where do the bull calves fit into the 4-H Club program? CLARENCE OLSON, North Dakota Agricultural College, Fargo.

E11. Some 4-H dairy record keeping results. H. A. WILLMAN, Cornell Univ., Ithaca, N. Y.

The dairy records program involves the use of 3 different record forms. The "completion report" is designed to give the county agent an annual record of all dairy projects as to size and breeds, the use of approved practices, and the participation of members in various project activities. The "young stock" and "cow production" records are set up to guide members in the feeding and management of their cattle. These records provide a basis of figuring costs and returns and surveying the results obtained. For instance, the need of more attention in growing yearling heifers was revealed in a study of the weights of 806 Holsteins.

A survey of the records entered in the 1948 and 1953 state contests showed a 201% gain in the records which were rated very good to excellent. The improvement in dairy record keeping during recent years probably should be attributed to such aids as *(a)* offering appropriate recognition to members for accomplishment and effort, *(b)* integrating instruction in record keeping with feeding and management lessons, and *(c)* promoting records largely on the basis of their value to the people who keep them.

E12. Suggestions for practical methods of evaluating pastures. GEORGE WERNER, Univ. of Wisconsin, Madison.

STUDIES ON AUREOMYCIN AND VITAMIN B_{12} SUPPLEMENTATIONS FOR DAIRY COWS. II. EFFECT ON PRODUCTION, COMPOSITION, AND VITAMIN B_{12} CONTENT OF THE MILK[1]

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Although it has been established that aureomycin (chlortetracycline) is beneficial to young calves, its value for mature ruminants has not been determined. Two reports *(6, 7)* in 1950 indicated adverse effects of feeding antibiotics to lambs and steers, but Rusoff *(17, 18)* in the same year reported beneficial effects of feeding aureomycin to ruminating calves 14 to 34 weeks of age. Subsequently, investigations with growing and fattening lambs *(11)*, fattening beef cattle *(16)*, mature dairy bulls *(20)*, and lactating dairy cows *(4, 10, 15, 20)* have indicated neither detrimental nor beneficial effect of feeding aureomycin to these species.

It has been reported by Rusoff and Haq *(19)* and by Williams and Knodt *(24)* that a vitamin B_{12} supplement did not produce any growth stimulation in young calves. Thus, the animals probably were obtaining their vitamin B_{12} from cow's milk, since several investigators *(2, 8, 9, 23)* have reported the vitamin B_{12} content of cow's milk to range from 2.6 to 7.6γ per liter.

The purpose of this study was to determine the effects of aureomycin and vitamin B_{12} supplementation of rations for lactating dairy cows with respect to milk production; the percentages of fat, total solids, solids-not-fat, total protein, casein, ash, calcium and phosphorus; the bacterial count and the vitamin B_{12} content of the milk. Two separate trials were conducted, the latter dealing specifically with increasing levels of vitamin B_{12} supplementation to the ration.

TRIAL I

EXPERIMENTAL

Fifteen lactating Holstein cows were obtained from the Louisiana State University dairy herd. These animals were divided into three comparable groups according to their age, stage of lactation, and gestation. Group I served as the control; Group II received the same basal ration as Group I and in addition 1% of aureomycin supplement[3] which supplied 130 mg. of aureomycin per cow daily; and Group III received the same basal ration and in addition 1% of vitamin B_{12} supplement[4] which supplied 0.83 mg. of vitamin B_{12} per animal

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[1] Data for this publication are taken from a dissertation submitted by M. O. Haq to the Graduate School of Louisiana State University in 1952 in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] Present address: E. Dacca, Pakistan.

[3] Courtesy of Lederle Laboratories Division, American Cyanamid Co., Pearl River, New York.

[4] Courtesy of Merck and Company, Inc., Rahway, New Jersey.

daily. All the animals received a 15.5% digestible protein grain mixture at the rate of 1 lb. for each 4 lb. of milk produced and had access to pasture. The grain mixture was composed of yellow corn meal, 30 lb.; crimped oats, 30 lb.; wheat bran, 20 lb.; cottonseed meal, 10 lb.; soybean meal, 10 lb.; oyster shell flour, 1%; defluorinated phosphate, 1%; and salt, 1%. The experiment was continued for a period of 60 days. Daily milk production records were maintained during the experimental period, and milk samples were collected at 2-week intervals for analysis. Standard methods of the A.O.A.C. *(3)* were used for determining percentage of fat, total solids, solids-not-fat, and protein. Ash, calcium and phosphorus values were obtained by the method of Morris *et al. (15)*. The vitamin B_{12} content of the milk was assayed microbiologically by the method of Skeggs *et al. (21)*. Standard bacteriological techniques were used in counting the bacteria present in the milks *(1)*.

RESULTS AND DISCUSSION

Milk production. The amounts of fat-corrected milk (FCM) produced by the different groups of animals are given in Table 1. The control group averaged 103.81 lb. FCM daily prior to the experimental feeding and 145.66 lb. during

TABLE 1
Average daily production of FCM

Group and ration fed	No. of animals	Pre-experimental	Experimental	Difference
		(lb.)	*(lb.)*	*(lb.)*
I Control	5	103.81	145.66	41.66
II Basal ration + 1% aureomycin supplement	5	92.09	131.80	39.72
III Basal ration + 1% vitamin B_{12} supplement	5	90.55	130.59	40.04

the experimental period of 60 days. Thus, there was an increase of 41.85 lb. FCM over the production for the initial period. Similarly, Group II (aureomycin-fed cows) increased its milk production by 39.72 lb. and Group III (vitamin B_{12}-fed cows) by 40.04 lb. over that of the initial period. The differences were not significant at $P < 0.05$. At the level fed, vitamin B_{12} or aureomycin supplementation of the ration did not influence the milk production of the animals. Similar results were obtained recently by Loosli and Warner *(14)* and Bartley *et al. (4)* in daily feeding of 100 mg. and 300-500 mg. of aureomycin, respectively, to lactating animals.

Composition of Milk. The percentages of fat, total solids, solids-not-fat, total protein, casein, ash, calcium, and phosphorus of the milks produced by the supplemented and control groups of cows are presented in Table 2. It can be observed that small differences are present for each constituent of the milks of the different groups, but these differences were not statistically significant at $P < 0.05$.

TABLE 2

Composition of the milks

Group	Fat (%)			Total solids (%)			Solids-not-fat (%)			Total proteins (%)		
	Initial	Exp.	Dif.	Initial	Exp.	Dif.	Initial	Exp.	Dif.	Initial	Exp.	Dif.
I (Control)	2.90	3.36	0.46	10.98	11.61	0.63	8.08	8.25	0.17	2.69	2.96	0.27
II (Aureomycin)	3.00	3.28	0.28	10.62	11.47	0.85	7.62	8.19	0.57	2.76	2.97	0.21
III (B_{12})	2.90	3.24	0.34	10.98	11.46	0.48	8.08	8.22	0.14	2.77	3.13	0.36
Group	Casein (%)			Ash (%)			Calcium (mg. %)			Phosphorus (mg. %)		
	Initial	Exp.	Dif.	Initial	Exp.	Dif.	Initial	Exp.	Dif.	Initial	Exp.	Dif.
I (Control)	2.03	2.16	0.13	0.65	0.69	0.04	98.43	103.20	4.77	92.05	102.90	10.85
II (Aureomycin)	1.91	2.15	0.24	0.67	0.68	0.01	93.33	96.85	3.52	92.05	101.65	9.60
III (B_{12})	1.92	2.22	0.30	0.67	0.69	0.03	95.37	101.29	5.98	94.77	103.44	8.67

Vitamin B_{12}. The vitamin B_{12} content of the milks from cows fed the vitamin B_{12} supplement and from those of the control group was assayed microbiologically. The control group averaged 1.77γ of vitamin B_{12} per liter of milk, and the vitamin B_{12} group averaged 1.69γ. These values are lower than the range of 2.6 to 12.4γ of vitamin B_{12} per liter of milk reported by other investigators *(2, 8, 9, 23)*.

Bacterial count. On the average, the bacteria content of the milk of the different groups varied from 9,000 to 12,700 per milliliter of milk. The average counts were 9,400, 12,200 and 12,700 per milliliter for the control, aureomycin, and vitamin B_{12} groups, respectively. Krienke *(13)* reported that milk from cows treated with aureomycin for mastitis was not suitable for cheese culture. The same result was reported by Bell *et al. (5)*, who mentioned higher concentrations of aureomycin in the milk after aureomycin therapy in mastitis. To observe whether or not the oral-fed aureomycin was being secreted into the milk, samples of milk were incubated at 35° C. These samples produced normal acid curd, signifying that the antibiotic was not secreted into the milk, or, if present at all, was of such low concentration that it did not cause any damage in the production of cheese culture.

TRIAL II

Since the vitamin B_{12} content of the milk was not increased by feeding a vitamin B_{12} supplement at a 1% level in the grain ration, a second experiment was conducted to find out whether or not higher amounts of vitamin B_{12} supplement would increase the vitamin B_{12} content of the milk.

EXPERIMENTAL

Twenty Holstein cows in late stage of lactation were divided into four comparable groups which were fed the same ration for a preliminary period of 7 days. Group I served as the control, Group II received the vitamin B_{12} supplement at a 2% level (1.66 mg. vitamin B_{12} per cow daily) in the grain mixture, Group III received the same supplement at a 3% level (2.49 mg. vitamin B_{12} per cow daily), and Group IV received the same supplement at a 4% level (3.32 mg. vitamin B_{12} per cow daily) for a period of 15 days. The animals also had access to grass-clover pasture. Daily milk production was recorded, and milk samples were collected from individual cows at the beginning of the experiment and at the completion of the experiment. The milk samples were analyzed for butterfat and vitamin B_{12} content.

RESULTS AND DISCUSSION

Milk production. The data on milk production are presented in Table 3. The control group averaged 66.90 lb. of FCM daily during the pre-experimental period and 61.20 lb. of FCM daily for the experimental period. During these same periods Group II averaged 65.70 and 60.10 lb. FCM daily; Group III, 73.20 and 67.50 lb.; and Group IV, 71.10 and 65.50 lb., respectively. The differ-

TABLE 3
Milk production of the various groups of cows

Group	No. of animals	FCM		
		Initial daily	Experimental daily	Difference
		(lb.)	*(lb.)*	*(lb.)*
I Basal	5	66.90	61.20	5.70
II Basal + 1.66 mg. Vitamin B_{12} daily	5	85.70	80.10	5.60
III Basal + 2.49 mg. Vitamin B_{12} daily	5	73.20	67.50	5.70
IV Basal + 3.32 mg. Vitamin B_{12} daily	5	71.10	65.50	5.60

ences in milk production among the groups of cows on various rations for these periods were not statistically significant at $P < 0.05$. Thus, a higher level of the vitamin B_{12} supplement (4%) in the grain ration did not influence milk production.

Fat production. The percentages of fat in the milk produced by the different groups of animals are shown in Table 4. The average percentages of butterfat in the milk of the different groups varied from 3.54 to 4.12%. The control group averaged 4.12% butterfat at the end of the experiment, whereas Groups II, III, and IV averaged 3.70, 3.70, and 3.70%, respectively. This difference was statistically significant ($P < 0.05$). In Trial I, a 1% level of the vitamin B_{12} supplement (0.83 mg. B_{12} daily) did not affect fat percentage. It appears that levels higher than a 1% vitamin B_{12} supplement might depress the butterfat production of the dairy cow.

Vitamin B_{12}. The vitamin B_{12} content of the milk from different groups of animals is presented in Table 5. The vitamin B_{12} content of the milk samples varied from 3.90 to 4.12γ per liter of milk at the completion of the experiment, whereas during the pre-experimental period the values varied from 1.71 to 1.80γ per liter of milk. The differences among the groups of animals in the vita-

TABLE 4
Percentage of butterfat in the milk

Group	No. of animals	Butterfat		
		Initial	Experimental	
			Range	Average
I Basal	5	3.80	3.6-4.4	4.12
II Basal + 1.66 mg. Vitamin B_{12} daily	5	3.54	3.4-3.9	3.70
III Basal + 2.49 mg. Vitamin B_{12} daily	5	3.54	3.1-4.2	3.70
IV Basal + 3.32 mg. Vitamin B_{12} daily	5	3.52	3.1-3.9	3.70

TABLE 5
Amount of vitamin B_{12} in the milk

Group	No. of animals	No. of samples	Initial	Experimental	
				Range	Average
			(γ/l)	*(γ/l)*	*(γ/l)*
I Basal	5	4	1.71	3.64-4.44	4.06
II Basal + 1.66 mg. Vitamin B_{12} daily	5	4	1.75	3.96-4.44	4.10
III Basal + 2.49 mg. Vitamin B_{12} daily	5	4	1.80	3.20-4.44	3.90
IV Basal + 3.32 mg. Vitamin B_{12} daily	5	4	1.75	3.96-4.42	4.12

min B_{12} content of the milk were not significant. The milk of all groups showed a significant increase in vitamin B_{12} content at the end of the experimental period. These increases in vitamin B_{12} are difficult to explain. Factors such as type of pasture, which might increase the cobalt content of the diet or change the activity of the rumen flora, stage of lactation, and season of the year might have exerted an influence on the vitamin B_{12} content of the milk, although Collins *et al. (9)* have reported that seasonal differences did not appear to affect the vitamin content of raw or pasteurized milk. The values obtained in this trial are slightly higher than those recently reported by Collins *et al.* of 2.6 to 3.8γ per liter of market milk but lower than the value obtained by Anthony *et al. (2)*, who reported an average of 7.6γ per liter for Jersey milk. It is concluded that the vitamin B_{12} content of cow's milk can not be increased by feeding a vitamin B_{12} supplement as high as a 4% level (3.32 mg. vitamin B_{12} per cow daily) in the grain ration.

SUMMARY

Studies were conducted with Holstein cows to determine the effect of an aureomycin supplement and a vitamin B_{12} supplement on milk production, on the composition of the milk, and on the vitamin B_{12} content of the milk.

Milk production and percentages of fat, total solids, solids-not-fat, total protein, casein, ash, Ca, and P of the milk were not influenced by feeding supplements of aureomycin (130 mg. daily) or vitamin B_{12} (0.83 mg. daily) at a 1% level in the grain ration.

The vitamin B_{12} content of the milk (1.69-4.12γ B_{12} per liter) was not influenced by increasing the level of vitamin B_{12} supplement from 1 to 4% (3.32 mg. B_{12} daily) in the grain ration. There was no change in milk production, but the fat content of the milk was slightly depressed when levels of vitamin B_{12} supplement were above 1% (0.83 mg. vitamin B_{12} daily) in the grain ration.

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A COMPARISON OF THE ABILITY OF CERTAIN EGG YOLK DILUENTS TO MAINTAIN OPTIMUM OSMOTIC CONDITIONS DURING THE STORAGE OF BULL SEMEN[1]

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Successful storage of semen depends upon control of physical and chemical factors in semen diluents which affect the survival of spermatozoa. Some of these — resistance factors, energy sources, electrolyte concentration and pH — have been extensively investigated and the results have been successfully applied to the improvement of semen diluents. However, one of the physical factors, osmotic pressure, has not been extensively investigated, particularly in reference to the influence of diluter constituents upon osmotic conditions during the storage period.

An optimum freezing point depression range of −0.44 to −0.61° C. for semen diluents was established by Pursley and Herman *(9)*. Swanson *(13)* observed that bovine spermatozoa are more sensitive to hypertonic solutions of sodium citrate than to hypotonic solutions and suggested that, as a result of glycolytic metabolism, an increase in the osmotic pressure of semen occurred during storage.

Generally, osmotic constants of biological fluids are determined by the standard cryoscopic method, or a modification (Salisbury *et al.*, *11*, and Crawford and Nicosia, *4)*; however, when the sample volume is small, as with semen, the volume requirements for a series of cryoscopic measurements on the same ejaculate become prohibitive.

Membrane osmometers requiring only a few drops of the fluids to be tested have been developed (Hansen, *6)*. But according to Aschaffenburg *(1)*, there are as yet no truly semipermeable membranes; therefore, membrane osmometers were not used.

Baldes *(2)* described a micro-method of measuring osmotic pressure using small thermocouples, and later Baldes and Johnson *(3)* described the construction of the osmometer. Fineman and McBaine *(5)* modified and improved the osmometer by forming the thermocouples from constantan and copper wire. Roepke *(10)* investigated factors affecting the accuracy of the method, and Lifson and Lorber *(8)* demonstrated both the theoretical and experimental existence of the steady state in less than a minute (40 seconds).

It is the purpose of the present paper to report the results obtained from an investigation, using the thermoelectric osmometer, of the changes in osmotic

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pressure of extended semen during storage and of the comparative ability of certain diluents used in the artificial insemination of dairy cattle to maintain an osmotic pressure within optimal limits throughout the storage period.

PROCEDURE

Calibration of osmometer. The thermoelectric osmometer used in this investigation was designed to incorporate ease of sample transfer. A detailed description will appear in a future publication by Smith, Mayer, and Herman *(12)*. The osmometer was calibrated at 25° C., using reference solutions of KCl. The plot of galvanometer deflections vs. calculated freezing point depressions of the reference solutions was a linear curve from which the freezing point of subsequent samples could be read from observed galvanometer deflections. Freezing point depressions of milk, egg yolk, ram semen, $NaHCO_3$, glucose, and sucrose of known molarity were obtained by use of the osmometer and compared with calculated or published values obtained from cryoscopic measurements. The values obtained with the thermoelectric osmometer were in agreement with those obtained from other sources and are presented in a publication by Smith, Mayer, and Herman *(12)*.

Collection and transport of semen. The semen used in this investigation was collected by use of the artificial vagina from healthy dairy bulls maintained as a part of the Missouri Station dairy herd and representing the Holstein, Jersey, and Guernsey breeds. Immediately after collection, the semen was cooled to 20° C., transferred to the laboratory, and pooled if necessary, and the composite sample was extended with the selected diluents for a particular storage trial.

Evaluation of the semen. The semen was evaluated for motility and freezing point depression. Motility was rated on a scale of 0 to 5 according to the method of Swanson and Herman *(14)* under 100× magnification at a temperature of 98° F. A motility rating of 5 on this scale represents a specimen with maximum motility, whereas a motility rating of 0 represents a specimen with no observable motility at 100× magnification.

Freezing point depression was determined with the modified Hill-Baldes thermoelectric osmometer.

Basic diluents used in this study. Six basic diluents (Table 1) were used in this study. One (524), which was developed in this laboratory from egg yolk, $NaHCO_3$, and glucose by Kampschmidt *et al. (7)*, differed from A only in the method of preparation and presence of antibiotics. Diluter A, prepared commercially on a large scale from lyophilized egg yolk, $NaHCO_3$, glucose, and antibiotics, was obtained in powder form and reconstituted with glass distilled water prior to use. Diluter 200, the sodium citrate diluter used extensively in the commercial practice of artificial insemination, contained equal quantities of egg yolk and 4.5% $Na_3C_6H_5O_7 \cdot 5\frac{1}{2}\ H_2O$ (sodium citrate) solution but contained no sugar, whereas diluters 635 and 400 differed from 524 only in the concentration of glucose and original freezing point depression of the diluent. Diluent 500, however, contained no glucose; its osmotic requirements were met by the addition of sucrose.

TABLE 1
Formulae of diluents

Diluter No.	Chemical components					
	Egg yolk	D.D.H_2O	5% Glucose	17% Sucrose	1.3% $NaHCO_3$	4.5% $Na_3C_6H_5O_7 \cdot 5\frac{1}{2}H_2O$
	(ml.)	*(ml.)*	*(ml.)*	*(ml.)*	*(ml.)*	*(ml.)*
635	10		20	20	10	
524	10		40		10	
500	10	14		26	10	
400	10	12.5	27.5		10	
200	20					20
A	Lyophilized product prepared commercially on a large scale					

Dilution, storage, and daily evaluation of semen. Semen, which had been evaluated for motility rating and freezing point depression, was mixed with one of the above diluters in the ratio of one part semen to nine parts diluter. Small vials of diluted semen were then placed in a container which held a large volume of water (400 ml.) and stored in a refrigerator at a temperature of 4 to 7° C.

RESULTS AND DISCUSSION

The data for motility rating and osmotic pressure determinations (freezing point depression) are given in Table 2. Data for freezing point depression are presented graphically in Figures 1 and 2.

Since three of the diluters studied have been or are now being used in the extension of semen for the artificial insemination of dairy cattle, the authors were interested in observing the comparative ability of these diluters to maintain the osmotic pressure of the extended semen within the optimal range for semen diluters as suggested by Pursley and Herman. Further, is maintenance of osmotic conditions within this optimal range of importance in controlling spermatozoan survival as measured by motility rating during a prolonged storage period?

Inspection of Figure 1 shows that diluter 524 maintains an osmotic pressure along the line of mean freezing point depression of bovine semen −0.55° C. and within rather narrow limits throughout the 10-day storage period; range in freezing point depression was −0.53 to −0.56° C. Diluter A, however, does not maintain the osmotic pressure within the same narrow limits, the range in freezing point depression being from −0.45 to −0.53° C. Diluter 524 is comprised of fresh egg yolk, glucose, and $NaHCO_3$ as the buffer salt, whereas diluter A is comprised of the same materials but is prepared commercially in the dried state and reconstituted in the laboratory by the addition of water. Both of these diluters maintained the osmotic pressure within the narrow limits proposed by Pursley and Herman as optimal for diluting mixtures. Since both diluters were superior in their capacity for maintaining motility throughout the 240-hour storage period, it is suggestive of a relationship between the capacity of a diluter to maintain osmotic conditions and to maintain motility.

The results obtained with diluter 200, which contained fresh egg yolk and sodium citrate but no sugar, substantiates this relationship. This diluter did

TABLE 2

Mean values of ΔTf (freezing point depression) and motility during storage of semen extended at dilutions of 1 in 10

Diluter	No. of trials		Hours storage										
			0	24	48	72	96	120	144	168	192	216	240
635	8	ΔTf	0.685	0.637	0.675	0.647	0.678	0.684	0.645	0.649	0.664	0.684	0.676
		Motility	2.8	2.6	2.1	2.1	2.0	1.8	1.8	1.5	1.2	0.9	0.6
524	10	ΔTf	0.561	0.542	0.551	0.558	0.547	0.546	0.534	0.545	0.529	0.561	0.536
		Motility	2.8	2.8	2.8	2.7	2.5	2.4	2.3	2.3	2.2	2.2	2.2
500	5	ΔTf	0.611	0.547	0.594	0.635	0.687	0.620	0.594	0.613	0.625	0.691	0.667
		Motility	3.0	3.0	2.4	2.2	2.0	2.0	1.8	1.4	1.2	0.8	0.8
400	7	ΔTf	0.492	0.478	0.455	0.440	0.488	0.438	0.462	0.474	0.455	0.509	0.421
		Motility	2.7	2.7	2.6	2.4	2.3	2.3	2.3	2.0	1.8	1.8	1.8
200	10	ΔTf	0.658	0.589	0.626	0.640	0.659	0.666	0.620	0.634	0.638	0.689	0.646
		Motility	2.9	2.9	2.7	2.5	2.4	1.8	1.8	1.6	1.3	1.1	0.8
A	16	ΔTf	0.466	0.486	0.532	0.527	0.494	0.450	0.476	0.457	0.472	0.457	0.498
		Motility	2.6	2.6	2.6	2.6	2.4	2.0	1.7	1.7	1.6	1.5	1.2

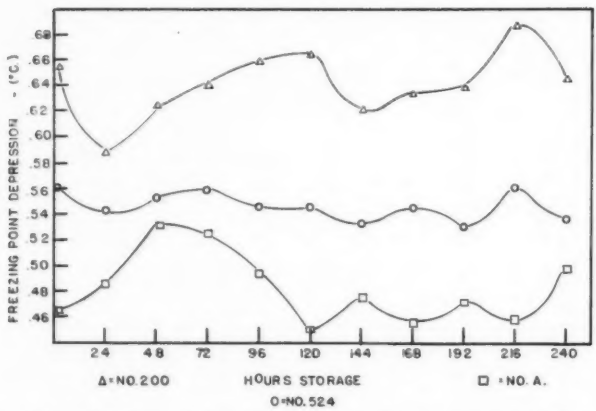

FIG. 1. Freezing point depression curves during storage of semen extended with diluents 200, 524, and A. Semen extended at a ratio of 1 in 10 (storage temp. 5° C.).

not maintain the osmotic pressure of extended semen within the narrow range maintained by diluters 524 and A throughout the entire storage period. The 24-hour value was within this range, but all other values were above the upper limit of the optimal range. Neither was this diluter (No. 200) as efficient in maintaining motility during storage, as shown in Table 2.

Differences in diluter constituents which could cause this observed variation in osmotic pressure occurring during storage of semen extended with these diluents have been considered. As previously stated, diluter A differed from 524 in that the egg yolk was lyophilized previous to the addition of the other ingredients and contained added antibiotics. Therefore, any difference in the shape of the freezing point depression vs. hours storage curve might be due to either

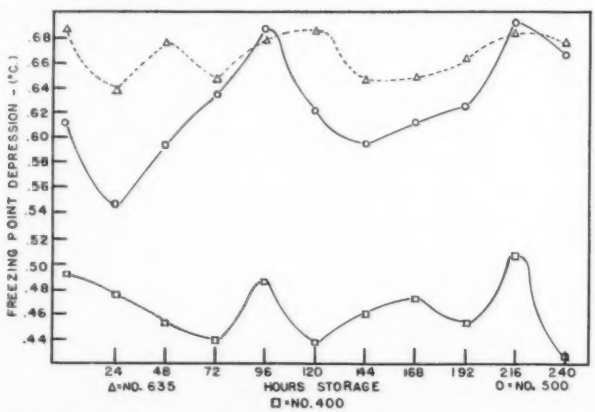

FIG. 2. Freezing point depression curves during storage of semen extended with diluents 635, 400, and 500. Semen extended at a ratio of 1 in 10 (storage temp. 5° C.).

of these differences. In fact, limited data have been obtained which would indicate that the differences are due to both lyophilization of the egg yolk and addition of antibiotics.

Diluter 200 differs from 524 and A in the buffer salt present, in the lack of added glucose, and in the egg yolk : buffer solution ratio. It may be that any of these differences could cause the rise in osmotic pressure observed in semen extended with diluter 200, during the first 120 hours of storage. However, the results obtained suggest that perhaps it is the lack of added glucose in the diluting media which is responsible for the rise in osmotic pressure observed.

Diluters 635 and 400 contained 20 and 27.5 ml. of 5% glucose, respectively, or about one-half as much as diluter 524. Since diluter 635 was prepared so that its original osmotic pressure was high, it maintained the osmotic pressure of semen extended with it above the limits established for optimum diluter performance. The hypertonic diluter 635 failed to maintain a motility rating of 2 for more than 96 hours of storage, whereas diluter 400 containing only slightly more glucose maintained a motility of 2 for 168 hours and one of 1.8 for the entire 240-hour storage period. The freezing point depression values with both diluters did not fluctuate to an excessive degree; diluter 635 maintained an osmotic pressure within the range of -0.64 to $-0.68°$ C. and diluter 400 within a range of -0.44 to $-0.51°$ C., both comparing well with that of diluter 524 in maintaining osmotic fluctuations within a narrow range. However, diluter 500, which contained no glucose, did not maintain osmotic pressure within a narrow range, nor did it maintain spermatozoan motility any better than diluter 200 which also contained no glucose. From this comparison it would appear that glucose aids in the maintenance of osmotic pressure within rather narrow limits throughout the storage period.

SUMMARY

Semen specimens from healthy dairy bulls were extended with six diluents, stored at 4 to 7° C. and examined daily for both motility and freezing point depression. One of these diluents, the egg yolk–citrate diluter, failed to maintain the osmotic pressure of the extended semen within the range suggested by Pursley and Herman as optimal for semen diluters. The other diluents used in commercial artificial insemination did maintain the osmotic pressure within these limits, and one of these, the egg yolk-glucose-$NaHCO_3$ diluter developed by Kampschmidt *et al.* *(7)*, maintained the osmotic pressure along the line of mean freezing point depression of semen, a point further strengthened by its superior motility maintenance during the storage period. It is suggested that added glucose aids in the maintenance of storage osmotic pressure within narrow limits and thus may have an influence upon the viability of bull spermatozoa during a prolonged storage period.

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FERTILIZATION RATES AND EMBRYONIC DEATH RATES IN COWS BRED TO BULLS OF DIFFERENT LEVELS OF FERTILITY[1]

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Variation in nonreturn rates of different bulls used in artificial insemination is not clearly understood. Theoretically, it must be due to variation in fertilization failure or in embryonic death or in both, but there seems to be little information on the relative importance of these two factors. The variation of sperm motility, in both raw and processed semen, of the bulls that are in active use in artificial insemination centers is too little and the known association between motility and fertility is too low for predicting whether fertilization failure is a major factor. Variation in nonreturn rate due to genital infections of the bull with consequent embryonic death is a possibility, but the incidence of recognized infections is too low to be adequate as an explanation. The possibility of other bull factors affecting embryonic death has been suspected but not proven. The object of this study was to determine the relative importance of fertilization failure and embryonic death in the variation of the fertility of bulls in use in artificial insemination centers.

MATERIALS AND METHODS

The chief objective in exercising other than random selection of the bulls studied was to get maximum variability in fertility. Holstein and Guernsey bulls in each of four studs were ranked each month from highest to lowest on the basis of their most recently available 60-90 day nonreturn rates. The immediately previous 1 or 2 months' nonreturn rates were then examined and the bulls eliminated that showed marked fluctuations from one month to the next. An attempt was made to select approximately equal numbers of high and low fertility bulls within the Guernseys and Holsteins of each stud. The two kinds of bulls in the early stages of the study were picked from the high and low fourths of the range of fertility; this could not be done in the latter part of the study without testing the same animals more than once, and it was deemed better to avoid this and test some bulls nearer to the average. As it developed, all of the bulls chosen as "high" had nonreturn rates of 67% or higher for the month which served as the basis of their selection, and all bulls chosen as "low" were below 67%. Final selection of bulls to be tested was limited to the animals from which semen had been collected for field use on the day a

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[2] Agents of the Bureau of Dairy Industry, U.S.D.A.

"tester" heifer came into heat. The most extreme untested high or low fertility bull ordinarily was chosen, but if more than one prospect desirable for test was available, a bull of low fertility was given preference over one of high fertility because of the tendency of breeding organizations to remove low testing bulls from service after a few months and the information on them thus to be lost. Any other selection practiced was made in an attempt to keep approximately equal the number of high and low fertility bulls within breeds and within studs.

The bulls selected and used in this study represent about half of the Holstein and Guernsey bulls in use in four artificial breeding organizations located in Wisconsin. It is not possible to get a clear-cut comparison of the variability between the bulls used and that of those not used. However, it would appear from a comparison of the variances of the nonreturn rates of the bulls used and a random sample of those not used the month of selection that variability was increased by selection by approximately 20%.

Tests of fertility of these bulls were made on 74 previously unbred grade heifers purchased especially for this purpose. Each heifer was inseminated with the semen from a test bull and slaughtered 3 to 5 days after the beginning of heat. The reproductive organs were removed, and the oviduct on the ovulating side was dissected from the rest of the genital tract. It was then flushed out with a physiological salt solution into a watch glass, and the recovered ovum was examined at 15× and usually also in a hanging drop preparation at 450×. The presence of approximately equal sized blastomeres, 4 to 32 in number, was accepted as evidence of fertilization; usually sperm were seen in the zona pellucida. If an egg was not recovered from a heifer, a retest was made with another heifer as soon as possible. Thus, a test required a recovered normal-appearing ovum from a female in which there was no apparent physical reason for fertilization failure. Of the 74 randomly selected heifers used in this study 65 (87.8%) had ova that either were fertilized or, if unfertilized, appeared normal and were in genital organs showing no condition which should prevent fertilization; four (5.4%) had only empty zona pellucidas. Multiple ovulations occurred in two (2.7%) of the heifers; in each heifer only one ovum was recovered and each ovum appeared to have abnormally shrunken cytoplasm. In one heifer (1.4%), no egg was recovered after repeated flushing; two (2.7%) of the heifers had hydrosalpinx that would have prevented fertilization, and one of these heifers also had bursitis.

The semen for test was taken directly from the processed semen in field use on the day of the test. All semen was diluted with either yolk-citrate or yolk-phosphate which contained antibiotics. No semen was used that was over 28 hours old (range 3½ to 28 hours), and ½ to 1 ml. of diluted semen (mean dilution 1:59, standard deviation 34) was deposited in the uterine body (usually) of the experimental heifer.

The nonreturn rates of each bull for the month of use were adjusted for the tendency of nonreturn rates to overestimate true pregnancies. Work of Barrett *et al. (2)* showed there was a discrepancy of 5.5% between 60-90 day

nonreturns and pregnancies, as determined by manual palpation in first service animals. Adler *(1)* found the same error of 5.5% between 60-90 day nonreturns and diagnosed pregnancies, and he presented data on individual bulls. Analysis of his data fails to show that there is any greater percentage of inseminated cows reported as nonreturns but actually not pregnant for bulls with low nonreturn rates than for those with high nonreturn rates ($r = 0.15$; $P > 0.05$). Therefore, in the present study the 60-90 day nonreturn percentage minus 5.5 has been used as the best estimate of actual pregnancies.

Further estimation was necessary to express the actual pregnancies as a percentage of potentially fertile cows; otherwise there would be an overestimation of embryonic death. What the percentage of first service cows may be that have permanent or temporary conditions preventing fertilization is open to conjecture.

On the evidence available from the tester females in this study, 12.2% was accepted as the best estimate of the percentage of inseminations that would fail because of abnormalities in the cow. If this estimate is too high, embryonic death rate will be underestimated.

Embryonic death, as estimated for a group of bulls, was based on the difference between the fertilization rate observed in the test heifers and the estimated actual pregnancies in potentially fertile cows.

$$ED = \frac{FR - \frac{(NR - 5.5)}{100 - 12.2}}{FR},$$

where ED is embryonic death rate, or the estimated percentage of fertilized ova that died in 60-90 days, FR is the percentage of recovered normal ova from test heifers that was fertilized, and NR is the unweighted average of the nonreturn rates of the different bulls during the month of test.

RESULTS

The 64 bulls on which fertilization tests were made consisted of 37 Holsteins and 27 Guernseys. The over-all nonreturn rates were 65.8% and 64.0% and the fertilization rates were 89.2% (33 out of 37) and 81.5% (22 out of 27) for the Holsteins and Guernseys, respectively. When tested by Chi square, the fertilization rates did not differ significantly ($P = 0.5 - 0.3$), and the nonreturn rates were so similar there is no real suggestion that there would be a difference in embryonic death.

As there appeared to be no significant breed differences in fertilization rates or in embryonic death, the breeds were pooled in further comparisons. Of the 64 bulls, 32 were selected originally as of high fertility and 32 as low. The average nonreturn rate for the high fertility bulls was 70.8% for the month of selection with a range from 67 to 77%. These bulls, in the month they were actually used, had an average nonreturn rate of 70.1% (a change of -0.7) and a range of 65 to 79%. In the month of selection the low fertility bulls had an

average nonreturn rate of 54.6% and a range of 31 to 66%. In the month of use their nonreturn average was 60% (a change of + 5.4) with a range of 40 to 71%. Analysis of variance shows a highly significant difference between the bulls selected as high and those selected as low in the tendency to change toward the mean.

The most accurate measurement of the field performance of the different bulls at the time of test was the nonreturn rate for the month of test. These rates were based in part on the same semen sample that was used to breed the test heifers and entirely on semen collected within a month of the time a heifer was bred. (It was not practical to separate the field results for the tested sample from the rest of the samples from a given bull in the same month.) The nonreturn rate of the bulls for the month of use was used as the independent variable, and the fertilization rate and embryonic death rate were used as dependent variables. The nonreturn rates were arrayed from highest (79%) to lowest (40%) and divided at the median (between 66 and 67%), and the associated results in the dependent variables were analyzed (Table 1). The

TABLE 1

Fertilization rates and estimated embryonic death rates — bulls classified as high or low

Classification	No. of bulls	Estimated pregnancies[a]	Recovered normal ova fertilized	Estimated fertilized ova dying before 60-90 days
		(%)	*(%)*	*(%)*
High (NR 67-79%)	32	74.5	100.0	25.5
Low (NR 40-66%)	32	61.2	71.9	14.9

[a] Nonreturn percentage minus 5.5 divided by percentage of cows having neither genital abnormalities nor ova defects, as estimated from experimental group of test heifers.

fertilization rate of the high fertility bulls was 32 out of 32 and for the low fertility bulls, 23 out of 32 (71.9%, $P < 0.01$). Embryonic death rate (here estimated as the per cent of fertilized ova that died) was 25.5 and 14.9 for the high and low fertility bulls, respectively. No entirely satisfactory method for determining statistical significance of the difference between these two embryonic death rates appears to be available. Analysis of variance of the angular transformation of the fertilization rates and the estimated pregancy rates, Rao *(5)*, indicated a significant interaction between fertility level of bull and the rate of pregnancy. This method tests the difference between two declines in fertility rather than between two proportions of the embryos that are lost, yet significance of the one may mean virtually the same as significance of the other.

The data as tested do not give evidence for more embryonic death for bulls of low fertility than for bulls of high fertility. In fact, a reverse hypothesis is supported. Such a finding could be explained on the basis that some selection has been exercised against high embryonic death and in favor of low fertilization rates in the keeping of low fertility bulls in service.

In an attempt to describe differences in fertilization rates and embryonic death rates more precisely, the bulls were divided according to their nonreturn

TABLE 2

Fertilization rates and estimated embryonic death rates — bulls classified as high, medium, and low

Classification	No. of bulls	Estimated pregnancies[a]	Recovered normal ova fertilized	Estimated fertilized ova dying before 60-90 days
		(%)	*(%)*	*(%)*
High (NR 70-79%)	22	75.8	100.0	24.2
Medium (NR 60-69%)	28	68.3	82.1	16.8
Low (NR 40-59%)	14	54.1	71.4	24.2

[a] Nonreturn percentage minus 5.5 divided by percentage of cows having neither genital abnormalities nor ova defects, as estimated from experimental group of test heifers.

rates the month of use into a high group with nonreturns of 70% and above (22 bulls), a medium group with nonreturns between 60-69% (28 bulls), and a low group with nonreturns of 59% and below (14 bulls, Table 2). Fertilization rates were 100% (22 out of 22), 82.1% (23 out of 28), and 71.4% (10 out of 14) for the high, medium, and low fertility bulls, respectively ($P < 0.05$). The estimated embryonic death rates (here estimated as the per cent of fertilized ova that died) for the three types of bulls were 24.2, 16.8, and 24.2%.

If potential young in dairy cattle is assumed to be one calf per breeding of first service animals, an estimate can be made of the different sources of reproductive loss in a population of first service cows. It would appear that only 59.6% of the potential young are present by 60-90 days after breeding. An estimate based on this study is given in Table 3 of the probable fate of the 40.4% potential young that are missing at 60-90 days. Of the young, 2.7% would be missing because of genital abnormalities in the cow, 9.5% would be missing as the result of defective or lost ova, 12.2% would be lost because of fertilization failure to which no cause other than sperm infertility could be ascribed, and an additional 16% would be lost because of death of the embryo, calculated in this case as a per cent of all potential young rather than as a per cent of fertilized eggs.

DISCUSSION

In work done thus far to analyze reproductive failures in cattle, considerable attention has been focused on the low fertility bull. Rottensten *(6, 7)* has reported a decreased conception rate of the return cows after service to low fertility

TABLE 3

Estimated fate of potential young

	(%)
Live embryos at 60-90 days:	59.6
Loss of potential young because of:	
Abnormalities of the reproductive tract	2.7
Defective ova	9.5
Fertilization failure, no apparent cause	12.2
Death of the embryo	16.0

bulls and has postulated that these bulls were carrying some "sterility producing factor" or infective agent that was causing embryonic death. Christian and Casida *(4)* observed that certain bulls at first service might affect the conception rates of bulls used on their return cows at later services. They further observed in bulls of satisfactory fertility a definite association between average length of the return intervals of their return cows and the fertility of those cows at second service, groups with long average return intervals tending to have low fertility when rebred. Salisbury *et al. (8)* have reported findings in substantial agreement with these. Thus, considerable evidence has accumulated suggesting embryonic death as making substantial contributions to the differences in nonreturns between bulls.

Another approach to the problem of reproductive failure in cattle has been made through the study of the repeat-breeder cow. Casida *(3)* reports the fertilization rate on 129 genitally normal cows of this type to be 60.7%, which is considerably below the over-all fertilization rate (85.9%) reported in this study (difference, 25.2%). Antibiotics were not used in the semen, however, for most of the repeat breeders. Estimated embryonic death rates (here estimated as the per cent of fertilized ova that died) were 59.4% for repeat breeder cows, compared to 21.0% (average of estimates for high and low fertility bulls) for the first service animals, or a difference of 38.4%. Thus, it appears that both embryonic death and fertilization failure are greater in the repeat-breeder cow than they are in the first service animal.

SUMMARY

Holstein and Guernsey bulls of different nonreturn rates in artificial insemination have been compared to determine their fertilization rates and to estimate their embryonic death rates. The 64 bulls for which fertilization tests were made on first service heifers had an average nonreturn rate of 65.1%, a fertilization rate of 85.9%, and an embryonic death date, estimated as the per cent of fertilized ova that died, of 21.0%. When the bulls were divided according to nonreturn rates into a high half (67-79%) and a low half (40-66%), they had fertilization rates of 100% and 71.9% ($P < 0.01$) and embryonic death estimates of 25.5% and 14.9% for the high and the low fertility bulls, respectively.

The differences were not significant between the fertilization rates of the Holsteins (89.2%) and the Guernseys (81.5%). The nonreturn rates of 65.8% and 64.0% were similar.

It appears from this study that approximately 40% of all potential young in dairy cattle are lost by 60-90 days after breeding. Their loss may be estimated as 3% due to genital abnormalities of the cow, 9% to defective ova, 12% to failure of fertilization resulting from undetermined causes, and 16% to death of the embryo, all calculated as percentages of the potential young producible by a female group.

ACKNOWLEDGMENTS

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FACTORS AFFECTING THE SENSITIVITY AND ACCURACY OF THE PHOSPHATASE TEST

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The phosphatase test for dairy products developed in the Dairy Products Research Laboratories (Sanders-Sager test, *14, 16, 17*) has been subjected to collaborative studies and adopted as an official method by the Association of Official Agricultural Chemists *(1)*. Gilcreas *(3)* reported collaborative results in which this method, in 135 examinations of pasteurized-milk Cheddar cheese, consistently yielded values indicating pasteurization of the milk and provided a reliable estimate of the heat treatment. Horwitz *(5)* reported collaborative results which showed that this method could be applied successfully to soft cheese.

Later, Kosikowsky and Dahlberg *(10, 11, 12)* and Kosikowsky *(7, 8, 9)* presented several phosphatase-test modifications, culminating in the development of a test called the Cornell Phosphatase Test *(9)*. They first presented a modified Kay-Graham phosphatase test for cheese *(10)*, using trichloroacetic acid to precipitate the proteins and extracting the phenol from the filtrate by means of ethyl ether. A later modification *(11)* included use of a sodium carbonate-bicarbonate buffer instead of sodium barbital, a combined trichloroacetic-hydrochloric acid precipitant, and optional use of BQC (2, 6-dibromoquinonechloroimine) instead of the Folin-Ciocalteu reagent. In other presentations *(7, 8, 9)*, in which the test was applied also to milk and other dairy products, ether extraction was omitted and BQC was used instead of the Folin-Ciocalteu reagent.

The chemistry and methodology of the tests and modifications mentioned above differ not only with respect to kinds and concentrations of buffers and precipitants, pH conditions, and conditions of color development, but also with respect to other factors of practical importance, such as temperatures and time periods of incubation. Such differences are important factors influencing the sensitivity and accuracy of results, particularly in applying the test to various dairy products.

The objectives of a phosphatase test include accurate measurement of the enzyme activity and correct interpretation of the results to indicate the adequacy of pasteurization. It has been well established by studies on the kinetics of enzyme reactions that enzyme activity may be accelerated or inhibited by various physico-chemical factors. The most accurate measure of enzyme activity is obtained during the early stages of incubation before inactivation effects become extreme. During the later stages the results tend to become more empirical and less accurate as a measure of the original activity.

For the purpose of definition in this paper the word "sensitivity" is used to refer to the quantity of phenol produced, i.e., the buffer or test yielding the most

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phenol is the most sensitive. The word "accuracy" is used to denote the correctness of the conclusions based on the test, these conclusions being dependent upon the correctness with which the enzyme activity is determined, the correctness and precision with which the liberated phenol is measured, and the soundness of the basis upon which the results are interpreted.

To obtain more information on the conditions necessary to yield the most sensitive, accurate, and reliable phosphatase test, the influences of certain chemical and physical factors on the activity of milk phosphatase were investigated. This paper presents that research and includes details of results summarized in published abstracts *(13, 15)*.

METHODS

Three methods were investigated and subjected to laboratory comparisons, as follows:

1. The Sanders-Sager *(14, 17)* method (designated S-S), employing a barium borate-hydroxide buffer, as described in Methods of Analysis of the A.O.A.C. *(1)*.

2. The "simple universal dairy products phosphatase test" *(7, 8)*, later called the Cornell Phosphatase Test *(9)*, employing a comparatively concentrated carbonate-bicarbonate buffer, 11.50 g. anhydrous sodium carbonate and 10.15 g. anhydrous sodium bicarbonate per liter, pH 9.8. Research was conducted also on details of earlier adaptations *(10, 11, 12)*, in which the liberated phenol was extracted with ether.

3. The Sanders-Hupfer modification (designated S-H), employing a carbonate-bicarbonate buffer differing from the Cornell buffer in $CO_3^=/HCO_3^-$ ratio, being more dilute and having a slightly higher pH. It was developed in an effort to increase the sensitivity and efficiency of a method employing a single incubation buffer for all dairy products and to study the use of carbonate-bicarbonate buffers and a trichloroacetic acid precipitant. It is similar to the other methods in general procedure but differs in the following respects: incubation buffer, 8.0 g. anhydrous sodium carbonate and 4.0 g. anhydrous sodium bicarbonate per liter, pH 10.1; precipitant, 42.0 g. trichloroacetic acid, 3.2 g. potassium oxalate, and 0.1 g. copper sulfate per 100 ml.; copper sulfate omitted in testing ripened cheese and fermented products; tests not heated after incubation; color development buffer, 35.0 g. sodium metaborate and 20.0 g. sodium chloride per liter.

Details applying to all methods. Four drops of chloroform was used in each overnight test. pH determinations were made with a Beckman glass-electrode meter at 25° C. or corrected to that temperature. The intensity of blue color in the tests was determined with a Klett-Summerson photoelectric colorimeter, employing a 610-mμ filter *(16)* for aqueous readings. When necessary, in comparisons of methods, the dilution procedure described earlier *(14, 17)*, with addition of more BQC, was employed in order to determine quantitatively all of the phenol present.

RESULTS AND DISCUSSION

Optimal pH for phosphatase activity. The optimal pH of incubation for the 1-hour test at 37.5° C. was found to be approximately 10.0 for the S-S and S-H methods, as shown in Figure 1 and as reported earlier *(16, 18)* for the S-S method. With the Cornell 1-hour method, a much flatter curve, with a broad optimal pH range, was obtained. Since the same type of buffer is used in the S-H and Cornell methods, the differences in shape and position of these two curves appear to be due to the concentrations of the buffers. Although the Cornell buffer yielded more uniform results, the phenol values being affected less by variations in pH, it yielded less sensitivity.

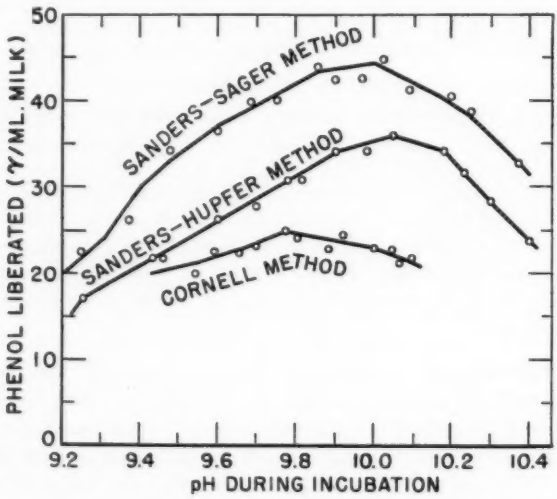

FIG. 1. Phosphatase activity in milk at different pH values, by three methods, 1-hour incubation period at 37.5° C. (1% of cow's raw milk in boiled milk).

The optimal pH for the 18-hour test at 32-35° C. was reported by Kosikowsky and Dahlberg *(12)* to be approximately 9.55 (optimal range, pH 9.40-9.70). In tests in these laboratories the optimal pH for incubation in 18-hour tests at 37.5° was found to be approximately 9.6 for each of the three methods.

The following data show the ranges of incubation pH values obtained by the three methods in tests on milk and also on Cheddar, Provolone, and Romano cheeses of various ages between two weeks and one year: milk — S-S method, incubation pH 9.87-10.10; Cornell method, 9.63-9.77; S-H method, 9.87-10.01; cheese — S-S method, 9.85-10.12; Cornell method, 9.42-9.65; and S-H method, 9.60-9.90. The S-S and S-H buffers did not yield incubation pH values optimal for both 1-hour and 18-hour incubation. The Cornell buffer yielded incubation pH values within the broad optimal pH range for 1-hour incubation and also within or close to the range for 18-hour incubation. However, as shown in

Figures 1, 2, and 3, the Cornell method was less sensitive than the S-S and S-H methods. Thus, none of the buffers yielded maximum enzyme activity under all conditions.

Comparison of relative sensitivities. Kosikowsky and Dahlberg compared the sensitivity of their earlier modifications *(10, 11)*, employing 18- to 24-hour incubation and ether extraction of phenol, with that of the S-S method, employing 1-hour incubation. They claimed greater sensitivity for their procedure, stating *(11)* that "The color in the present procedure has no interfering off-colors and the pH, the buffer and the longer time of incubation induce maximum enzyme activity." However, analyses here, by the ether extraction procedure, of test samples with known amounts of phenol yielded recovery of only 50 to 70% of the phenol, resulting in false negative tests.

The relative sensitivities of the S-S method, the Cornell universal method, and the S-H method were determined with various periods of incubation at 37.5° C. Typical results are shown in Figures 2 and 3. The sensitivity of each method increased with increases in the period of incubation. The sensitivity in 1-hour tests on milk and also on Cheddar cheese was greatest with the S-S method and least with the Cornell method. The sensitivity in 18-hour tests on milk was greatest with the S-S method and least with the S-H method (Figure 2). In 18-hour tests on cheese it was greatest with the S-H method, less with the S-S method, and least with the Cornell method (Figure 3).

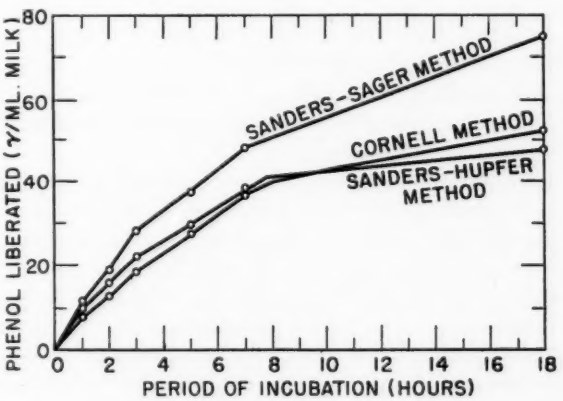

FIG. 2. Rates of enzymatic hydrolysis in phosphatase tests by different methods at 37.5° C. (averages, 0.3% of raw milk in boiled milk).

Thus these results indicate that the Cornell method does not yield conditions producing "maximum enzyme activity." The higher phenol values yielded in most cases with the S-H buffer — approximately half as concentrated as the Cornell buffer — suggested that the enzyme activity was diminished by the greater concentration of the buffer used in the Cornell method. Sensitivity differences noted in the three methods may be ascribed to nonoptimal pH con-

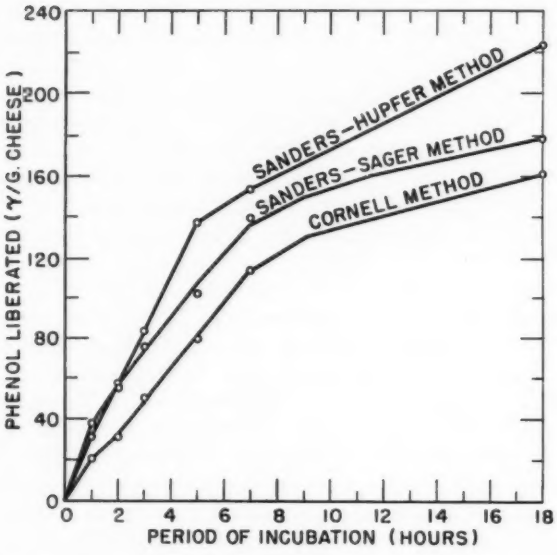

FIG. 3. Rates of enzymatic hydrolysis in phosphatase tests by different methods at 37.5° C. (averages, 0.6% of raw-milk Cheddar cheese mixed with pasteurized-milk Cheddar cheese, 8 months old).

ditions, enzyme inactivation by high alkalinity (especially with long incubation), inhibition by $CO_3^=$ and HCO_3^- ions, high ionic strength of buffers, effect of barium in precipitating certain inhibitory ions, and decrease of optimal temperature with increased period of incubation.

Further research showed that the lower sensitivity of the S-S method compared with the S-H method, in 18-hour tests on cheese (Figure 3), was caused by the fact that the pH during incubation was not within the optimal range. The optimal pH for 1-hour tests is approximately 10.0, but that for 18-hour tests is approximately 9.6. By diluting the appropriate S-S incubation buffer with an equal volume of water, the incubation pH was reduced to a range of 9.40 to 9.75 with Cheddar cheese samples of different ages up to 1 year old. In 18-hour test comparisons, with the buffer thus diluted, the S-S method yielded higher phenol values than either of the other methods.

Effects of buffer concentration. The influences of various concentrations of the Cornell and the S-H buffer on the phosphatase activity in 18-hour tests on samples from one lot of milk are shown in Figures 4 and 5. The undiluted S-H buffer yielded greater sensitivity than the undiluted Cornell buffer. With both buffers, large increases in sensitivity were produced by employing dilutions as great as ten-fold (1 part buffer + 9 parts water), and even with the less concentrated S-H buffer the incubation pH was not reduced below the optimal range for 18-hour tests. The results show that the Cornell buffer is much more concen-

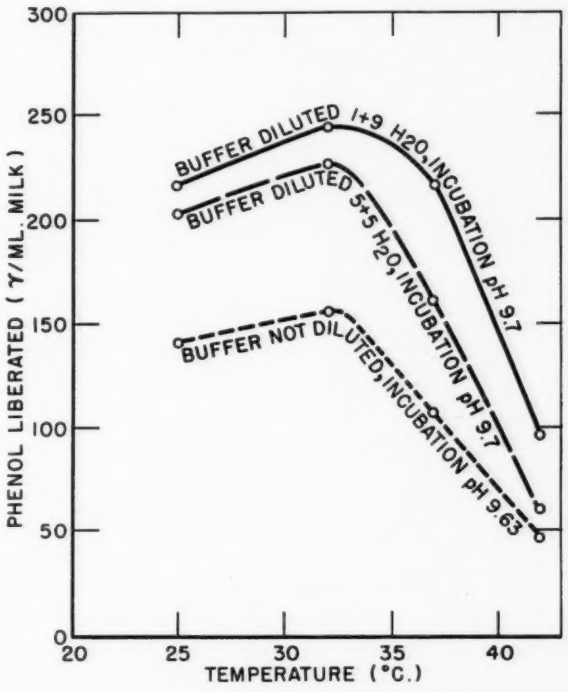

FIG. 4. Effects of buffer dilution and of incubation temperature on phosphatase activity in Cornell 18-hour method (1% of raw milk in boiled milk).

trated than necessary, and even the S-H buffer is somewhat more concentrated than necessary, to yield maximum sensitivity in testing milk.

Results of 18-hour tests on samples of the same milk, with various concentrations of the S-S incubation buffer, are shown in Figure 6. Both dilutions of 25-11 buffer (25.0 g. Ba $(OH)_2 \cdot 8H_2O$ and 11.0 g. H_3BO_3 used in preparing 1 l.) gave greater sensitivity than either the 25-11 or the 26-11 buffers undiluted; but at 32° C. the 25-11 buffer diluted 5 + 5 with water (incubation pH 9.9) was more sensitive than the 25-11 buffer diluted 1 + 9 (incubation pH 9.4).

Influence of incubation temperature. In the Cornell method *(9)* the temperature of incubation was specified as 32 to 37° C. (for 18 to 24 hours). This range seemed too wide for quantitative, reproducible results. Accordingly, tests with 18-hour incubation periods were conducted by the three methods, including modifications in which the regular buffers were diluted. The results (Figures 4, 5, and 6) show marked differences in activity between incubation at 32 and at 37° C. In these methods with the usual buffers and 18-hour incubation, the activity peaks were at or near 31 to 32° C. (or below), and the results at 37° C. were much lower. With carbonate-bicarbonate buffers (Figures 4 and 5), the

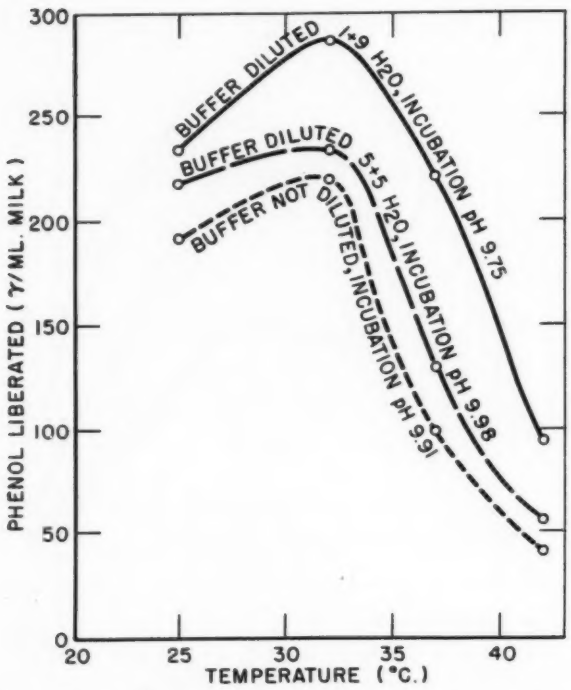

FIG. 5. Effects of buffer dilution and of incubation temperature on phosphatase activity in Sanders-Hupfer 18-hour method (1% of raw milk in boiled milk).

difference in activity between 25 and 32° C. was relatively slight, but the activity decreased greatly with change in temperature from 32 to 37° C. Thus a temperature range of 32 to 37° C. is shown to be too wide to yield reproducible results, and 32° is preferable to 37° C. for maximum sensitivity with 18-hour incubation.

Figure 6 shows effects of rather wide differences in pH, brought about by various dilutions of the S-S buffer, upon the optimal incubation temperature in the 18-hour test on milk. With a 1 + 9 dilution of the buffer and incubation pH 9.4, maximum activity occurred with incubation at 37° C. With a 5 + 5 dilution and incubation pH 9.9, maximum activity occurred at 32° C. With a more concentrated buffer (26-11, for testing cheese) and incubation pH 10.28, maximum activity occurred at 24° C. These results show that when the pH is comparatively high during incubation the temperature should be comparatively low in order to obtain maximum sensitivity.

Protein precipitation. In tests employing carbonate buffers, difficulty was sometimes encountered with some samples because of cloudiness in the color development step. Cloudiness occurs when an excess of carbonate is present and

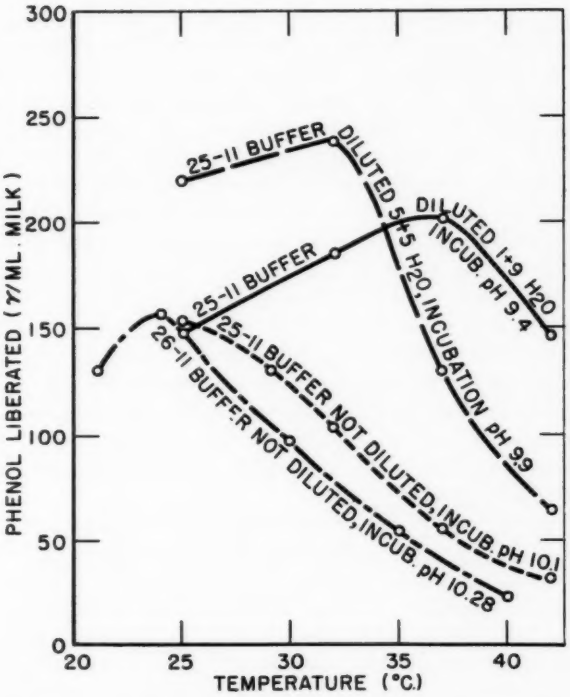

FIG. 6. Effects of buffer dilution and of incubation temperature on phosphatase activity in Sanders-Sager 18-hour method (1% of raw milk in boiled milk).

particularly in cheese tests in which considerable calcium is present. In the Cornell method *(9)*, to avoid cloudiness, addition of sodium hexametaphosphate as a sequestrant is specified. Sodium carbonate is then added, shifting the pH to a range of 9.6-9.8 for color development. In the S-H modification, to avoid cloudiness, the calcium concentration in the filtrate is reduced by adding potassium oxalate in the trichloroacetic acid precipitant. Excess carbonate is avoided by using sodium metaborate (buffered with sodium chloride) as a color development buffer. This buffer shifts the pH to approximately 9.3-9.4, which has been found to be more suitable than a higher pH for maximum blue color development with minimum off color *(2, 4)*.

Effectiveness of BQC. Direct comparisons were made to determine the effectiveness of BQC in developing blue color. Test filtrates were prepared from milk and also from ripened Cheddar cheese by each of the usual methods except that the disodium phenyl phosphate was omitted and known quantities of phenol were added. In seven comparisons, the following are averages of values for quantities of phenol developed in each test, with 50γ of phenol present, by addition of exactly 0.04 ml. of fresh BQC solution (approximately equal to 2 drops): S-S

method 31.6γ (pH of color development 9.3-9.4); Cornell universal method 26.4γ (pH 9.7-9.8); and S-H method 28.4γ (pH 9.3-9.4). The results indicated that BQC is more efficient in the S-S method than in either of the other methods. More blue color in the S-S method may be explained by lower concentrations of interfering ions and by the lower, more favorable pH. In all the methods the results showed that under these conditions more blue color developed in milk filtrates than in cheese filtrates — generally from 25 to 33γ in the former and from 15 to 22γ in the latter. Less blue color in cheese filtrates may be explained by the presence of more interfering compounds resulting from ripening.

In these laboratories direct comparisons were made of amounts of interfering yellow color in controls, in connection with 27 milk tests and 38 Cheddar cheese tests, with exactly 0.04 ml. of BQC solution added in each test. Averages of the 65 control readings for each method, determined by means of a Klett-Summerson photoelectric colorimeter at 610 mμ wavelength, were as follows: S-S method 40, Cornell universal method 38, and S-H method 43 scale divisions. These values for this instrument would be equivalent to 3.3, 3.2, and 3.6γ of phenol in the test. The results show little or no significant difference in control values among the three methods employed.

In a later publication it was stated that the Cornell method *(9)* yielded considerably lower control blank values than the S-S method. However, the later modification specifies a smaller quantity of control sample than the S-S method, which would reduce control readings. The desirability of reducing the quantity of control sample is questionable since the purpose of a control is to determine the amount of color yielded by a sample of the same size without milk phosphatase, including possible phenolic contamination or the presence of microbial phosphatase.

Linearity of results. One of the important requirements of a reliable, quantitative method for phosphatase is that the quantities of phenol liberated and detected in the tests be linearly proportional to the amounts of enzyme or the proportions of raw milk in the samples. A lack of linearity indicates either that liberation of phenol by the enzyme has been limited or that all of the liberated phenol has not been determined accurately. In collaborative work by Horwitz *et al. (6)* the S-S method with aqueous photometric reading was found to rank higher than the Gilcreas-Davis and Scharer methods with respect to quantitative linearity.

To determine more fully the relationships between test results and actual enzyme concentrations, raw milk was added to boiled milk in various concentrations and these dilutions were tested by the S-S method with 1-, 4-, and 18-hour incubation periods. The 1- and 4-hour tests were incubated at 37° C. The 18-hour tests were incubated at 32° C. to provide more nearly optimal conditions. The results with concentrations of 0 to 10% of raw milk are shown in Figure 7. In 1-hour tests, linear (straight-line) results were obtained with more than 20% of raw milk in the samples (not shown in Figure 7). The line began to curve at about 30%. In 4-hour tests, it began to curve between 5 and 10%. In 18-hour tests, a straight line was obtained up to about 1%. These results indicate that

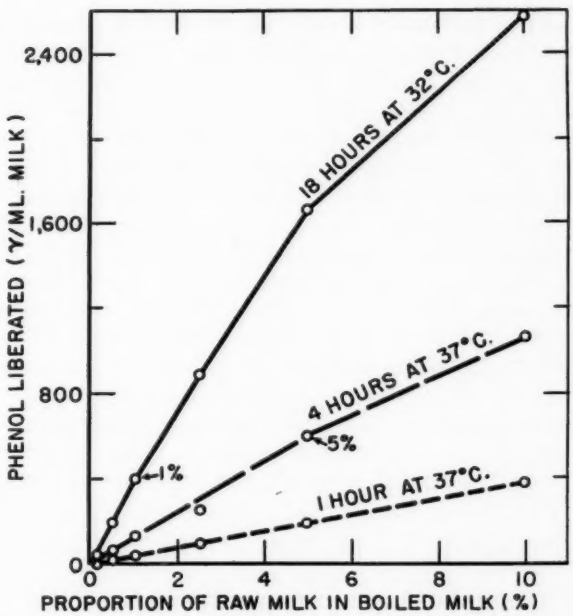

FIG. 7. Ratio of phenol liberated to phosphatase present as affected by period of incubation (25-11 barium borate-hydroxide buffer diluted 5 + 5 with water; 0.2% disodium phenyl phosphate substrate; incubation pH 10).

the values obtained in an 18-hour S-S test are valid for measuring accurately the activities of small concentrations of enzyme, as in under-pasteurized milk.

Effect of added phosphate. Inhibition of phosphatase by certain negative ions, including phosphates, was mentioned earlier *(18)* in connection with the desirability of using a barium buffer to precipitate those ions. Data presented by Kosikowsky and Dahlberg *(12)* showed that addition of 3% disodium phosphate or sodium citrate to Cheddar cheese did not cause inhibition in the presence of a carbonate-bicarbonate buffer substrate with overnight incubation. Experiments were conducted in these laboratories, with the three methods, on three lots of Cheddar cheese made from pasteurized milk and with 1% of raw cream added to the cheese samples. Two per cent of disodium phosphate was added to a portion of each lot, and 2% of sodium citrate was added to another portion (both chemicals on an anhydrous basis).

The average results with 1-hour incubation showed no inhibition due to added phosphate in the S-S method and approximately 14% inhibition in the Cornell and the S-H methods. The results with 18-hour incubation showed no inhibition due to added phosphate in the S-S method (incubation pH 10.1), 17% inhibition in the Cornell method (incubation pH 9.55), and 21% inhibition in the S-H method (incubation pH 9.8). In the 18-hour S-S tests, with the incubation

buffer diluted 5 + 5 with water (incubation pH 9.6), the average results with samples without added phosphate were higher than those obtained by any of the other methods, and approximately 25% inhibition occurred with added phosphate. The addition of citrate did not produce significant inhibition in any of the methods. These results indicate that the use of the usual S-S buffer containing barium aids in reducing the inhibition of added phosphate and that comparative inhibition caused by added phosphate may be greater with buffers of relatively low ionic concentration.

Criteria for pasteurization. The criteria for pasteurization in the 1-hour S-S method *(14)* are 2γ per 0.5 ml. of milk and 3γ per 0.25 g. of Cheddar and most other kinds of cheese. The criteria in the Cornell method *(9)* are 2γ per 0.5 ml. of milk or per 0.25 g. of cheese for the 1-hour test, and 5γ per 0.5 ml. of milk or per 0.25 g. of cheese for the 18- to 24-hour test. Although Figures 2 and 3 show decreases in velocity of the enzyme reaction with length of time, the quantity of phenol produced in an 18- to 24-hour test is comparatively large, and the results show that if a criterion of 2γ is used for a 1-hour test, then a criterion of 5γ for an 18- to 24-hour test is too low. To determine the ratios, data were tabulated showing results with various incubation periods at 37-38° C., on six samples of milk each tested by two persons, and on 10 samples of Cheddar cheese. Results for 4-hour incubation are included. A 4-hour test may be advantageous because of greater sensitivity than obtained by 1-hour incubation and because the results of tests can be obtained the same day. The average ratios of respective phenol values for incubation periods of 1, 4, and 18 hours were as follows: for milk — S-S method, 1:2.8:6.2; Cornell method, 1:2.9:6.4; and S-H method, 1:2.7:4.8; for cheese — S-S method, 1:2.4:5.6; Cornell method, 1:2.7:7.5; and S-H method, 1:2.8:7.4. These results, summarized for both products, indicate that with a pasteurization criterion of 2γ for a 1-hour test the criterion for a 4-hour test should be approximately 5γ, and for an 18-hour test approximately 12γ. The results for a 24-hour test would be even higher. These results indicate that a criterion as low as 5γ for an 18-hour test might impose an undue burden upon plant operators, requiring more heating than specified in present pasteurization standards. Pasteurization experiments under precisely controlled conditions would be necessary to establish accurate criteria.

In view of the effectiveness of BQC in producing sufficient blue color to be easily detected in tests containing 2γ of phenol, the need for an overnight test yielding as much as 12γ, or even 5γ, is questionable, especially in view of the extra time involved.

SUMMARY

In studies of effects of buffer concentrations, pH values, and incubation temperatures during different time periods on sensitivity and accuracy of phosphatase tests, direct comparisons were made by means of the Sanders-Sager test, the Cornell test, and a modification referred to as the Sanders-Hupfer test. In 1- and 18-hour tests on milk and 1-hour tests on cured Cheddar cheese, the S-S

method yielded the highest values. In 18-hour tests on cheese, the S-H modification yielded the highest values and the Cornell method the lowest.

The optimal pH for 18-hour incubation in these methods was found to be 9.5-9.6, as shown by Cornell workers using an 18-hour test, rather than pH 10 of the 1-hour S-S and S-H methods. In 18-hour tests on cheese, the S-S method with a diluted buffer, incubation pH about 9.6, yielded the highest values. With the usual buffers in each test the optimal temperature for 18-hour incubation was found to be lower (31-32° C.) than for 1-hour incubation (37-38° C. or slightly above). With relatively high pH during incubation the optimal temperature for 18-hour tests was relatively low. Buffers of relatively great ionic concentration retarded the enzyme activity but the concentration of carbonate-bicarbonate buffers did not affect the optimal temperature.

For maximum enzyme activity it is necessary to correctly adjust buffer concentration, pH, and incubation temperature. With buffers not too concentrated, it was found possible to compensate for pH variations by adjusting the incubation temperature correspondingly, thereby increasing the sensitivity and accuracy of a "universal" test.

The quantity of phenol liberated in the 18-hour S-S method at 32° C. was linearly proportional to raw milk concentration up to approximately 1%.

The average 18-hour test values on milk and cheese for the three different methods were more than six times the 1-hour values. To correspond with a 2γ criterion for the 1-hour test, the data suggest that the criterion for pasteurization for the 18-hour test should be approximately 12γ.

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THE USE OF WHITE MUTANTS OF *PENICILLIUM ROQUEFORTI* IN CHEESE MAKING[1]

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The use in cheese making of mutants of microorganisms prepared by induced mutation is, so far as the authors are aware, a new idea. Knight *et al. (4)* have suggested the possibility that white mutants of *Penicillium roqueforti* might be of value in cheese manufacture. This is a report on some of the experiments that have led to the development of a new cheese in which one of these mutants is used.

METHODS

The mutants of *Penicillium roqueforti* used in this study (UW-12 and UW-15) were prepared from the parent Minnesota strain P-108-2 by irradiation with ultra violet light by Knight *et al. (4)*.

The samples for the analytical determinations were taken from one half of a cheese which was ground and thoroughly mixed. Volatile acidity values *(6)*, pH *(5)*, and the acid number of the cheese fat *(2)* were used as approximate measurements of ripening progress. The cheese fat was obtained by a 4-hour ethyl ether extraction of 25 g. of cheese in a Soxhlet extraction apparatus. Salt *(7)* and moisture *(1)* determinations were made routinely. The cheeses were examined periodically by the authors for flavor, description of the mold present, body, and texture.

In the first experiments the two mutants and their parent strain were compared. Minnesota Blue cheese curd *(3)* from a vat of University mixed herd raw milk was divided into four portions. One portion of the curd was left uninoculated. The second portion was inoculated with mutant strain UW-15, the third portion with mutant strain UW-12, and the fourth portion with the parent strain P-108-2. These portions of curd were then hooped, salted, and ripened in the usual manner. This procedure was repeated in a second trial except that there were no cheeses made from uninoculated curd.

Other experiments were set up to compare one of the mutants and its parent strain in cheese made from milk processed in different ways. One half of a lot of mixed herd raw milk was homogenized at a temperature of 110° F. and 2,500 p.s.i. pressure. The other half was not heated or homogenized. These two milks were made into cheese following the procedure used in the manufacture of Minnesota Blue cheese *(3)* except that 1 day elapsed between manufacture and salting instead of 3 to 5 days. One half of the curd from each batch was inoculated with UW-12 and the other half was inoculated with P-108-2. These experiments were repeated with pasteurized milk (143° F. for 30 minutes) instead of raw milk.

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RESULTS

A comparison of the appearance of the mold, flavor, body, and texture of cheese made from uninoculated curd, curd inoculated with mutant strain UW-15, curd inoculated with mutant UW-12, and curd inoculated with the parent strain, is presented in Table 1. The cheeses inoculated with UW-12 and P-108-2 were not contaminated with other molds; the uninoculated cheese and the UW-15 inoculated cheese were contaminated.

TABLE 1

The appearance of mold, flavor, body, and texture of cheese inoculated with several strains of P. roqueforti

Age	Mold strain	Description of mold	Flavor	Body and texture
(days)				
27	*(a)* none	none present	acidy	firm, not broken down
	(b) UW-15	none visible	acidy	firm, not broken down
	(c) UW-12	white, well sporulated	acidy	firm, not broken down
	(d) P-108-2	blue, well sporulated	acidy	firm, not broken down
97	*(a)* none	scattered blue mold contamination	acid, bitter	firm, not broken down
	(b) UW-15	slight invasion, sparse white mold, V. slight blue mold contamination	acid, bitter	firm, not broken down
	(c) UW-12	excellent white mold, no contamination	acid, sl. bitter, sl. Blue cheese	firm, not broken down
	(d) P-108-2	normal blue	acid, sl. bitter, sl. Blue cheese	firm, not broken down
162	*(a)* none	yellow and blue mold contaminants	musty	not broken down
	(b) UW-15	white mold well distributed, sl. blue mold contamination	acidy, very slight Blue cheese	not broken down
	(c) UW-12	excellent white mold growth, well distributed	slight Blue cheese, clean	slight breakdown, more than *a*, *b*, or *d*
	(d) P-108-2	excellent blue mold growth	slight Blue cheese	slight breakdown, normal for age
229	*(a)* none	yellow and blue contaminants	very musty	not broken down
	(b) UW-15	slight blue mold contamination, good white mold	Blue cheese like	not broken down
	(c) UW-12	no contamination, excellent white mold	good Blue cheese, not pungent or peppery, clean, pleasant, best of group	slight buttery, more breakdown than *a*, *b*, or *d*
	(d) P-108-2	no contamination, excellent blue mold	raw mold, sl. Blue cheese	more breakdown than *a* or *b*

The flavor, body, and texture of the UW-12 cheeses were superior to the other cheeses. The Blue cheeses behaved normally, whereas the UW-15 cheeses were slow in ripening. The flavor of the uninoculated cheeses was musty, and the body was firm and crumbly.

Analytical data on these cheeses at 8 months of age (marketable age for Minnesota Blue cheese) are presented in Table 2. The moisture and sodium chloride contents were comparatively uniform among the cheeses examined. The pH was higher in the UW-12 cheese, suggesting the possibility of more proteoly-

TABLE 2

The moisture, salt, pH, volatile acidity, and acid number values of 8-month-old cheese inoculated with several strains of P. roqueforti

Mold strain	Moisture	NaCl	pH	Volatile acidity				Acid number
				Residue	Water soluble	Water insoluble	Total	
	(%)	*(%)*		*(ml N/10 NaOH/10 g cheese)*				*(ml N/10 KOH/1 g)*
(a) none	44.57	4.0	5.68	0.36	2.55	1.98	4.60	2.85
	44.61	4.0		0.36	2.60	1.98	4.65	2.80
(b) UW-15	43.48	4.4	5.72	0.39	3.57	1.42	5.09	2.81
	43.98	4.3		0.38	3.43	1.53	5.05	2.87
(c) UW-12	43.57	4.3	5.90	0.35	1.35	1.15	2.56	2.12
	43.23	4.3		0.30	1.35	1.20	2.56	2.15
(d) P-108-2	44.02	4.3	5.57	0.30	1.40	0.82	2.23	1.96
	44.11	4.3		0.35	1.45	0.85	2.36	1.94

sis in this cheese. The total volatile acidity and acid number were higher in the uninoculated cheese *(a)* and the cheese inoculated with UW-15 *(b)* at this time. Whether the other cheeses *(c* and *d)* had previously higher volatile acid values and higher acid numbers was not determined.

A repeat of the first experiment revealed similar results. The judges' comments on these cheeses at 4 and 6 months of age are presented in Table 3. The cheeses inoculated with UW-12 mold strain had better flavor and softer body and texture at 4 and 6 months than did the other mutant or the parent strain. The faster ripening observed for all cheeses, compared with cheese of the first experiment, may have been due partly to a higher moisture, which averaged 44.82%.

TABLE 3

Mold appearance, flavor, body, and texture of 4- and 6-month-old cheese inoculated with several strains of P. roqueforti

Mold strain	Age of cheese	Description of mold	Flavor	Body and texture
	(mo.)			
UW-12	4	excellent white mold, slight mold contamination at the entrance of the punch holes	mild Blue cheese-like flavor, more pronounced than the other two	more body breakdown than the other two
UW-15	4	excellent mold growth	Blue cheese-like slight raw mold	slight body breakdown
P-108-1	4	excellent mold growth and color	raw mold, slight acid	firm, slightly crumbly
UW-12	6	no contamination in punch holes—excellent mold growth	delicious Roquefort-like	soft, creamy body, smooth texture, slightly crumbly
UW-15	6	contamination in punch holes, excellent mold growth	raw mold	slightly crumbly
P-108-2	6	fair mold distribution	raw mold, acidy	crumbly

TABLE 4

A comparison of the volatile acidity and pH of 4- and 6-month-old cheese inoculated with several strains of P. roqueforti

Mold strain	Age	Volatile acidity				pH
		Residue	Water soluble	Water insoluble	Total	
	(mo.)	*(ml N/10 NaOH/10 g. cheese)*				
UW-12	4	0.37	2.67	1.01	3.75	6.05
UW-15	4	0.57	1.65	1.10	3.03	6.00
P-108-2	4	0.25	1.47	0.74	2.17	5.70
UW-12	6	0.42	2.18	1.04	3.33	5.90
UW-15	6	0.25	1.30	0.81	2.05	5.60
P-108-2	6	0.27	1.13	0.60	1.69	5.60

A comparison of the volatile acidity and pH values of 4- and 6-month-old cheeses from the second experiment is shown in Table 4. The white mold cheeses at both ages had a higher volatile acidity, the highest being the cheese inoculated with UW-12. The pH of the UW-12 cheese also was slightly higher. The reason for the drop in volatile acidity in the 6-month-old cheese compared to the 4-month-old cheese may have been the conversion of the acids to nonacidic compounds. The reason for the drop in pH of the UW-15 cheese is not known. The pH values of the other cheeses were about the same at 6 months as at 4 months.

On the basis of these two experiments the mutant strain UW-12 was chosen for further study. Experiments were carried on to compare the mutant and its parent strain in cheese made from milk processed in different ways, as previously indicated.

Data obtained from a typical experiment involving cheese made with raw milk and raw homogenized milk, one half of each lot being inoculated with a mutant strain and the other half with the parent strain, are presented in Part A of Table 5. The data presented are from cheese at 4 months of age. The normal white mold cheese had a greater pH than did the others. This cheese lacked typical Blue cheese flavor but had excellent body and texture. The cheeses made from the homogenized raw milk were typical of much of the Blue cheese sold on the market today. The white mold cheese made from normal milk was thought to be superior to the Blue cheese made from the same milk.

In Part B of Table 5 are presented typical data obtained with pasteurized milk. This milk was treated in a similar manner to that described in Part A. The white mold cheese made from the normal pasteurized milk had a higher pH than the normal Blue cheese, but with the homogenized milk cheese there was no difference. The flavor of the "normal" white mold cheese was slightly different from that of the Blue cheese. It did not develop a typical Blue cheese flavor but had a clean, mushroom-like flavor. However, the white mold cheese made from homogenized milk had a mild Blue cheese flavor along with the slight mushroom flavor. The white mold cheese made from the normal pasteurized milk had a softer body and a smoother texture than did the Blue cheese. Even though a single experiment is reported here, numerous subsequent experiments using milk

TABLE 5

A comparison of pH, flavor, body, and texture of 4-month-old cheese made with various types of milk and inoculated with several strains of P. roqueforti

Part A

	Raw milk			
	Normal		Homogenized	
	White mold	*Blue mold*	*White mold*	*Blue mold*
pH	6.42	5.6	5.42	5.68
Flavor	flat lacks flavor clean	raw mold flavor	Ketone sharp, clean peppery	normal commercial Blue cheese slightly soapy
Body and texture	buttery	sl. buttery	very buttery	buttery

Part B

	Pasteurized milk			
	Normal		Homogenized	
	White mold	*Blue mold*	*White mold*	*Blue mold*
pH	6.38	5.47	6.08	6.05
Flavor	clean, mild lacks typical Blue cheese flavor, mushroom flavor	clean Blue cheese, sl. raw mold	pleasing very sl. soapy sl. mushroom- like flavor	sl. soapy sl. raw mold flavor
Body and texture	buttery	buttery	buttery	buttery

which had been subjected to various treatments yielded comparable results. No contamination by other molds has been observed in the interior of the UW-12 white mold cheese. Occasionally, a slight invasion of dark colored mold has been noted at the surface of the cheese at the punch marks. In no instance has this contamination been great enough to cause a serious defect in appearance.

DISCUSSION

It is apparent from the results presented and from other observations made on cheese manufactured by accepted methods in commercial cheese plants that the characteristics of the white mold cheese can be influenced greatly by the methods used to treat the milk and by the cheese-making process itself.

On the basis of body and texture observations, it appears that white mutant UW-12 is more proteolytic than either mutant UW-15 or the parent strain. Cheese of a marketable age typically has a soft buttery body which has excellent spreadability as contrasted with a firmer, more crumbly body of typical Blue cheese of the same age. This superior body characteristic and the distinctive color, along with the wide variation in intensity of desirable flavor made possible by various manufacturing procedures may make this cheese attractive for commercial production.

Currently, one commercial plant is making acceptable mild-flavored cheese from pasteurized, homogenized milk inoculated with the UW-12 mutant. This cheese, known as "Nuworld," is ready for the market at 90 to 120 days, and consumer reaction has been favorable.

The fact that cheese-making or ripening organisms can be altered genetically so that they have a different appearance and possess a different biochemical behavior is a new approach to cheese manufacture. There are many other possibilities of producing new products by the use of stable mutant microorganisms.

SUMMARY

Two white mutants of *Penicillium roqueforti* have been compared with their parent strain in a series of cheese-making experiments. One of these mutants was found to be superior to the other.

Comparative data on flavor, body, texture, appearance, acid number, total volatile acidity, and pH have been presented.

Excellent cheese can be made with a white mutant of *Penicillium roqueforti*. Its color, body, texture, and comparatively milder flavor are enough different from Blue cheese to constitute a new cheese, which may be classed within the semi-soft, mold-ripened group of cheese.

ACKNOWLEDGMENT

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FREE FATTY ACID CONTENT OF FRESH MILK AS RELATED TO PORTIONS OF MILK DRAWN[1]

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The free fatty acid content of fresh milk, expressed as acid degree, has been established by obtaining the fat by churning and then titrating the purified fat with alcoholic base to the phenolphthalein end point. By utilizing such a procedure, workers have found the acid degrees of mixed fresh milk and milk from individual cows to be between 0.1 and 0.9 *(3, 4)*. The accuracy of these findings now may be questioned, since Johnson and Gould *(7)* demonstrated that churning results in the loss of some free acids. Later, an extraction procedure for obtaining the fat was developed by these investigators which yielded higher free acid values because of a more complete recovery of the fatty acids *(8)*. The use of the extraction procedure to establish the true normal free fatty acid content of fresh milk fat is in part the basis of this paper.

Only limited attention has been given to measuring the free acid content of freshly drawn milk from individual cows and how this may be related to fat content of the milk, differences of the individual cow, and stage of lactation. In addition, the free fatty acid content of different portions of milk obtained during milking has not been determined. This survey study was made to obtain this information.

EXPERIMENTAL PROCEDURE

The extraction method of obtaining the fat for free fatty acid determinations was essentially that of Johnson and Gould *(8)*, except that the size of sample was reduced from 125 g. to 60 g., and the quantity of reagents was adjusted accordingly, i.e., 25 ml. of 95% ethanol followed by a mixture of 40 ml. of ethyl ether and 60 ml. of Skellysolve F. A comparison was made between this procedure and that proposed by Hollender *et al. (6)*. Lactic acid was found to cause errors in the results of the latter method; consequently, it was not studied further.

The free fatty acid content of the fat was determined by the titration procedure of Breazeale and Bird *(1)*, slightly modified. One gram of fat was dissolved in 10 ml. of Skellysolve F and 5 ml. of absolute ethanol and titrated to the phenolphthalein end point with 0.01 *N* KOH in absolute ethanol by using a 5-ml. microburette. Results are expressed in terms of acid degree: milliliters of 1 *N* base required to titrate 100 g. of fat.

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The Babcock test was employed to determine the fat content of the milk.

Measurements were made of the fatty acid content of three different portions of milk drawn from each of the cows selected for the study. The first portions (about 500 ml.) were obtained by hand milking, so that each quarter contributed about an equal quantity. This first sample was designated as Portion A. The cow was then milked by machine until little milk remained in the udder. An aliquot sample was taken from the milking machine portion (Portion B). The milk remaining in the udder after machine milking (usually 400 to 600 ml.) was removed by hand (Portion C).

All samples were collected in glass bottles, placed immediately in an ice bath, and cooled rapidly to about 5° C. Free fatty acids were determined within 1 hour after obtaining the first milk sample.

EXPERIMENTAL RESULTS

Acid degrees of portions of milk drawn. For five consecutive days, milk from the evening milkings of two cows (a Holstein and a Jersey, both advanced in lactation) was obtained in the portions indicated (A, B, and C) and analyzed for free fatty acids. The results are presented in Table 1.

These data reveal a definite relationship between the portion of milk drawn and the acid content; the first drawn milk always being appreciably higher in acid degree than the middle portion, and the last portion being generally the same or slightly higher than the middle portion. When Portion A is considered in comparison to the other portions, a definite relationship is apparent between fat content and free fatty acid content; this portion was decidedly lower in fat and higher in free fatty acids than the other portions.

TABLE 1

Acid degree and fat content of various portions of milk from a Holstein and a Jersey cow for five consecutive days

Stage of lactation	Portion A		Portion B		Portion C	
	Fat	Acid degree	Fat	Acid degree	Fat	Acid degree
(days)	*(%)*		*(%)*		*(%)*	
			Holstein cow			
256	1.4	2.40	3.3	1.08	4.1	1.42
257	1.7	2.40	3.3	1.83	3.6	2.23
258	1.4	2.12	3.5	1.04	3.4	1.70
259	1.4	2.04	3.0	1.55	3.3	1.04
260	1.1	1.67	2.2	1.26	3.6	1.11
Average		2.13		1.35		1.50
			Jersey cow			
321	2.9	2.75	4.3	1.18	6.8	1.24
322	2.8	1.39	5.6	0.93	6.2	0.87
323	2.8	1.07	6.1	0.77	5.8	0.79
324	2.3	1.25	5.5	0.81	4.8	0.89
325	1.9	2.08	4.6	0.79	5.0	0.84
Average		1.71		0.90		0.93

The results in Table 1 indicate considerable daily variation in the fatty acid content of a specific portion of milk. The greatest variation is noted in Portion A for both the Holstein and the Jersey, whereas Portion B exhibited the least variation. This may be expected, since the middle portion represents about 85% of the total milking. When the entire day's milking was considered, the acid degree varied from 1.11 to 1.53 for the Holstein and from 0.80 to 1.31 for the Jersey. These fatty acid values are considerably higher than those reported previously for freshly drawn milk, except those reported by Dunkley *(2)* for cream.

Acid degrees during different stages of lactation. To study the changes in acid degree of different portions of milk obtained during the lactation period, four cows (two Holsteins and two Jerseys) which had been in milk from 92 to 157 days were selected from the University dairy herd. Four series of analyses were conducted on the milk of each of the four cows during various subsequent stages of lactation covering a period of about 160 days. The investigation was initiated during the summer while the cows were on pasture and was terminated during the winter. The data are presented in Table 2.

TABLE 2

Acid degrees of various portions of milk from Holstein and Jersey cows as related to stage of lactation

Cow	Days of lactation	Portion A		Portion B		Portion C	
		Fat	Acid degree	Fat	Acid degree	Fat	Acid degree
		(%)		*(%)*		*(%)*	
Holstein	122	0.6	2.16	3.2	0.78	8.0	0.62
	127	0.3	6.53	3.0	0.77	7.0	0.62
	150	0.3	2.76	2.3	0.85	5.7	0.92
	279	1.4	1.51	4.0	0.74	7.7	0.92
Holstein	92	0.9	1.03	3.0	0.85	5.1	0.71
	97	0.4	4.95	4.0	0.78	4.9	0.66
	120	0.4	2.27	2.8	0.84	4.1	0.87
	249	1.3	2.80	3.5	1.41	3.6	1.52
Jersey	155	4.0	0.71	5.4	0.55	6.2	0.65
	160	2.0	1.03	4.1	0.63	9.2	0.51
	183	2.5	0.99	5.2	0.59	10.4	0.41
	312	7.1	0.56	7.9	0.66	9.3	0.61
Jersey	157	2.0	1.08	3.9	0.54	8.7	0.46
	162	3.8	0.95	5.5	0.68	6.6	0.76
	185	1.4	1.41	3.7	0.60	9.3	0.57
	314	2.2	1.73	5.7	0.67	9.0	0.70

These results show wide variations in the free fatty acid content of the milks obtained at the different times. The variations in the milk from an individual cow appeared to be as great as those related to the breed or stage of lactation. No definite trend of fatty acid content with the progress of lactation was evident.

The general relationship between portion of milk drawn, fat content, and acid degree, noted in connection with the data in Table 1, is further borne out by the data in Table 2. The first portion of milk was higher in fatty acid content and lower in fat content. Unusually high acid degrees were found in Portion A

for the milk from Holstein 1 at 127 days and Holstein 2 at 97 days. However, none of the fresh milk obtained in this study was rancid in flavor at the time of analysis.

Additional studies were conducted in which milk from eight cows of five major breeds was analyzed for free fatty acids. Four of the cows were early in lactation (from 24 to 44 days) and four were relatively late in lactation (from 231 to 355 days). The data are shown in Table 3.

TABLE 3

Acid degrees of various portions of milk obtained from eight cows at different stages of lactation

Cow	Days of lactation	Portion A		Portion B		Portion C	
		Fat	Acid degree	Fat	Acid degree	Fat	Acid degree
		(%)		*(%)*		*(%)*	
			Early lactation				
Ayrshire	24	0.9	1.53	3.5	0.60	6.0	0.54
Ayrshire	28	0.7	1.57	4.4	0.45	10.5	0.50
Guernsey	37	1.1	1.48	3.8	0.52	8.5	0.54
Guernsey	44	1.2	1.63	3.6	0.57	7.9	.....
			Late lactation				
Guernsey	231	2.9	0.51	5.1	0.52	5.9	0.41
Brown Swiss	249	2.1	1.15	3.9	1.58	6.1	1.26
Holstein	287	2.8	0.65	5.3	0.92	6.9	0.92
Jersey	355	6.0	0.36	5.7	0.41	10.7	0.35

These results are somewhat similar to those presented in Table 2. However, it may be noted that the free fatty acid content of Portion A from the cows early in lactation was appreciably greater than that of the same portions of milk from the four cows late in lactation. Also, although the fatty acid content of Portion A was appreciably higher than that in the other portions for the cows early in lactation, this difference did not exist for the various portions from the four cows late in lactation. However, the data for these four cows are not consistent with the data presented in Tables 1 and 2 for other cows in late lactation and emphasize the importance of individual cow variation.

The relationship of the fat content to free fatty acid levels is emphasized in the results for Portion A. In the samples obtained for the early lactation trials, the fat content of Portion A ranged from 0.7 to 1.2% and the acid degrees from 1.48 to 1.63. In contrast, the late lactation milks ranged from 2.15 to 6.0% in fat and from 0.36 to 1.15 in acid degrees. For this latter group there was less variation in the free fatty acid content of the different portions; free fatty acid values of Portion A generally were less than those of Portion B and not appreciably different from those of Portion C.

Total milk yield for each cow was determined and compared to acid degree. The data are not presented in detail because no relationship was observed between milk yield and acid degree.

DISCUSSION

Several observations may be made as the result of this study. First, the results reveal that the fatty acid content of milk will vary widely between different cows and between successive daily milkings from the same cow. Second, the data indicate a tendency for the fatty acid content of portions of the milk to decrease as the milking proceeds to completion, although this is subject to variation. Third, by using an extraction procedure for obtaining the fat for fatty acid titration, acid degree results obtained appear to be generally higher than those which have appeared in literature.

The results are conclusive in showing that all freshly drawn milk contains measurable quantities of free fatty acids. The question exists as to the cause of these free acids. There are at least two possibilities: that in the milk secretion process there are always some fatty acids which are not combined into glycerides; that lipase action is occurring in the udder which brings about variable amounts of lipolysis of the formed glycerides. Kelly *(9)* has shown the presence of lipases in mammary gland tissue.

The causes for the normal variations in the free fatty acid content of fresh milk from individual cows have not been revealed by this study. The breed of cow, fat test, and milk yield do not appear to be factors. Physiological conditions are doubtless involved, since observations have been made that rancid milk was obtained from cows with abscessed ovaries *(10)*.

The results do not lend any direct evidence to the belief that milk from cows late in lactation may have a higher free fatty acid content at milking time than milk from cows early in lactation development. It may be significant to note that in certain cases the foremilk (Portion A) from cows late in lactation was higher in fat content and lower in free fatty acids than the foremilks from cows in early lactation.

The decrease in the fatty acid content of the three successive portions of milk, which generally occurred, was usually especially marked between Portion A and the two other portions. For all samples analyzed the average acid degrees of the first, middle, and last portion were 1.77, 0.83, and 0.82, respectively. The values ranged from 0.36 to 6.53 for Portion A, 0.41 to 1.83 for Portion B, and from 0.41 to 2.23 for Portion C. The magnitude of the difference between the acid degree of Portion A and the other portions apepars to be, at least partly, related to the fat level. Usually the fat content of the first portion was about 1.0% or less, and the free fatty acid content was comparatively high. However, in certain cases of late lactation, when the fat content of the Portion A was above 2%, the difference in free fatty acid content of the various portions was not as great as for cows whose milk in Portion A had a fat content of less than 2.0%.

The high free fatty acid content of the first portion drawn may be related to fat content, to fat globule size, or to the fat-aqueous ratio, which would result in a larger percentage of water-soluble acids per unit of fat being measured in a low-fat than in a high-fat portion. This is indicated by the work of Johnson and Gould *(8)*, who showed the fatty acid content of low-fat milk to be appre-

ciably higher than that of high-fat products when the fat was obtained for titration by the extraction procedure.

Another possible explanation of the high free fatty acid content of the first portion may be that this portion had the most opportunity to be affected by lipase action in the udder. This first portion is mostly in the milk cistern at the time of milking and represents the milk which was present in the udder the longest time. Microscopic examination revealed that the fat globules in the first portion were smaller. Therefore, more surface area per unit weight of fat, together with a longer time for lipolysis than the other portions, would favor more lipase action on the fat of the first portion.

From the standpoint of the acid degrees reported in this study, if the middle portion is assumed to be representative of the milk supply, the acid degrees of 34 samples of milk range from 0.41 to 1.83, with an average of 0.82. Since these are higher than published values, it would appear desirable to consider establishing more logical normal values for the free fatty acid content of fresh milk fat based on the use of extraction procedures for obtaining the fatty material for titration.

SUMMARY

Various portions of milk as drawn from different cows, representing five breeds and different periods of lactation, were analyzed for free fatty acid content by utilizing an extraction procedure to obtain the fat for titration. Three portions were obtained, with about 85% of the milk drawn being in the second portion. Measurable quantities of free fatty acids were found in all samples analyzed. Generally, the trend was for the first-drawn milk to be the highest and the last-drawn milk to be the lowest in acid degree. However, for individual cows late in lactation there was a tendency for all three portions of milk to exhibit more nearly the same acid degree. When the fat content was nearly that of normal milk no correlation was noted between fat content of the milk and acid degree of the milk fat. The same portions of milk from individual cows, i.e., first, second, or third, were found to vary widely in acid degree values from day to day. Also, milk from different cows varied daily in free fatty acid content.

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A HIGH ROUGHAGE SYSTEM FOR RAISING CALVES BASED ON THE EARLY DEVELOPMENT OF RUMEN FUNCTION. V. SOME EFFECTS OF FEEDING AUREOMYCIN WITH DIFFERENT RATIOS OF HAY TO GRAIN[1]

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Numerous reports showing that aureomycin acts as a growth stimulant when included in the ration of young dairy calves have appeared in the literature *(1, 3, 4, 5, 7, 16, 19, 22, 25, 30, 31, 32)*. Although many theories have been advanced, the mode of action of the growth-stimulating effect of aureomycin and other antibiotics is not well understood *(8)*.

In this experiment the purpose was to determine the effect of feeding aureomycin to calves being raised on the high roughage system *(15)* with different ratios of hay to grain. The criteria selected to measure the effects of aureomycin and the proportion of hay to grain were growth, feed consumption and digestion, efficiency of feed utilization, nitrogen metabolism, the volatile fatty acid content of rumen juice, the thiamine and riboflavin content of both rumen juice and urine, blood sugar, and changes in rating of certain indicator rumen microorganisms.

Growth responses to aureomycin feeding obtained by various investigators have varied somewhat in magnitude depending on the conditions of the experiments. McKay *et al. (22)* reported a 16% increase in gain; Loosli and Wallace *(19)*, 22%; and Rusoff *et al. (31)*, 10-20% from aureomycin supplementation. Murley *et al. (25)* and Bartley *et al. (4)* showed that the efficiency of feed utilization was improved when aureomycin was fed. On the other hand, McKay *et al. (22)* noted no statistically significant differences in efficiency of feed utilization due to aureomycin feeding.

Rusoff *et al. (32)*, using the indicator method *(27)*, concluded that aureomycin feeding did not change the usual microflora of the rumen in calves fed an all-vegetable protein calf starter free choice up to 6 lb. daily for Jerseys and 7 lb. for Holsteins, with medium grade lespedeza hay. Bartley *et al. (5)*, using direct microscopic examinations, found no consistent differences in rumen microorganisms between aureomycin-fed and control calves. Neumann *et al. (26)* noted that the total numbers of rumen microorganisms were unaffected but that some varieties failed to appear in Gram stains of aureomycin-fed heifers. Chance *et al. (12)* observed a marked drop in the total number of bacteria in steers when aureomycin was fed.

Bell *et al. (6)* observed decreased crude fiber digestion and anorexia from feeding 0.6 g. of aureomycin HCl per day to steers. Similar results have been

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reported for sheep *(13, 35)*. Results of digestion experiments indicate that protein digestion and nitrogen metabolism are not materially affected by aureomycin feeding *(3, 25)*.

Chance *et al. (11)* found that riboflavin levels in the rumen were lower after feeding aureomycin.

Catron *et al. (10)* observed a greater increase in blood glucose levels after oral glucose administration in aureomycin-fed pigs than in the controls.

EXPERIMENTAL PROCEDURE

Twenty-four Jersey calves (12 ♂, 12 ♀) were divided into three groups of eight each. After a 3-day colostrum feeding period, whole milk was fed at the rate of approximately 1 lb. per 10 lb. of birth weight until the animals were 7 weeks old. Each group of eight calves was fed good quality, second cutting, mixed alfalfa-clover-timothy hay at one of the following hay to grain ratios: 4:1, 3:2, 2:3. The hay was fed free choice from the third day of age, allowing a 10-15% refusal. The amount of grain fed each calf on the various hay to grain ratios was governed by the amount of hay being eaten. The grain concentrate mixture consisted of 73 lb. of ground shelled corn and 27 lb. of soybean oil meal. The total protein of the concentrate mixture was approximately 18%.

All calves were rumen-inoculated as previously described *(27)* at the end of each of the first 6 weeks. At the end of the first 4 weeks, 250,000 U.S.P. units of vitamin A in capsule form were given each calf.

Four calves in each of the three groups (5 ♂, 7 ♀) were fed aureomycin and four calves in each group (7 ♂, 5 ♀) served as controls. Fifteen mg. of aureomycin in the form of Aurofac 2A (Lederle) was fed daily in the milk to 7 weeks of age. From the end of the seventh week to 12 weeks of age the aureomycin was added to the grain mixture, the amount being adjusted so that each calf received 20 mg. of aureomycin per pound of total dry feed (hay plus grain) consumed daily. The average amounts of aureomycin consumed during the eighth week were 29.5, 34.0, and 54.0 mg. per day by the calves fed the 4:1, 3:2, and 2:3 hay to grain ratios, respectively. Corresponding amounts consumed during the 12th week were 78.8, 90.3, and 94.6 mg. per day.

All calves were weighed at weekly intervals and withers height was measured at birth and at 12 weeks of age. Records of daily feed consumption also were kept.

Blood samples were drawn from the jugular vein at weekly intervals and analyzed for glucose by the method of Somogyi *(34)*.

Rumen samples were taken by stomach tube at 4, 6, 9, and 12 weeks of age. Total steam volatile fatty acids were determined and chromatographic separations were made to determine the proportion of acetic, propionic, and butyric acids in the rumen juice. The chromatographic separations were made by the method of Buhlen *et al. (9)* as adapted to rumen juice by Linke *(17)*. Ratings of the rumen microorganisms *(27)* were made on the same samples by using wet preparations for protozoa and Gram stains for the indicator bacteria. The

thiamine content was determined by the thiochrome method and riboflavin microbiologically according to the procedures described by the Association of Vitamin Chemists *(2)*.

The male calves were continued on experiment during the 13th week and placed in metabolism stalls, where feed consumption data and 24-hour urine and feces samples were collected. These samples were used to determine digestibility of dry matter, cellulose *(14)*, and protein and nitrogen retention. Also, excretions of thiamine and riboflavin in the urine were determined, which, along with the rumen juice level, gave a relative indication of the synthesis of these vitamins.

After the aureomycin feeding period (12 weeks for females and 13 weeks for males), 21 of the calves, including both the controls and those fed aureomycin, were continued on experiment to 26 weeks of age and fed either a 2:1 or a 4:1 ratio of hay to grain. Ten calves, five controls (2 ♂, 3 ♀) and five previously fed aureomycin (2 ♂, 3 ♀), were assigned to the 4:1 hay to grain group. Eleven calves, five controls (3 ♂, 2 ♀) and six previously fed aureomycin (2 ♂, 4 ♀), were assigned to the 2:1 hay to grain group. The calves were divided so that approximately equal numbers, fed at the three different hay to grain ratios during the first 12 weeks, were in the control and aureomycin groups.

Daily records were kept of hay and grain consumption. As in the first phase of the experiment, hay was fed free choice and the amount of grain fed was governed by the amount of hay being eaten. These calves also were weighed at weekly intervals, and withers height was measured at 26 weeks of age.

RESULTS AND DISCUSSION

The growth, feed consumption, and efficiency of feed utilization data for the first 12 weeks, comparing the aureomycin-fed calves with the controls, at the three hay to grain ratios, are summarized in Table 1. Regardless of the ratio of hay to grain fed, the aureomycin-fed calves made greater gains both in body weight and in withers height. The aureomycin-fed calves also consumed more total feed and calculated TDN and NE and utilized their feed more efficiently, as measured by calculated TDN and NE per pound of gain. The differences in growth among the various ratio groups were significant ($P < 0.01$), as were also the differences in height at withers ($P < 0.05$). However, efficiency of feed utilization was not greatly influenced by the ratio of hay to grain fed, as indicated by TDN and NE per pound of body weight. When the mean gains were adjusted for TDN intake (Table 1), no differences in growth were found among the three hay to grain ratios fed, and the differences due to aureomycin feeding were the same regardless of the ratio of hay to grain fed. The differences due to aureomycin feeding were statistically significant ($P < 0.01$).

Despite the advantage in favor of the aureomycin-fed calves at 12 weeks of age, there was a reversal during the period of 12 to 26 weeks, when these calves gained less in both body weight and withers height and utilized their feed less efficiently than the calves that had been controls on both hay to grain ratios (2:1 and 4:1), as shown in Table 2. Data showing the growth of the control calves

TABLE 1

Effect of aureomycin on feed consumption, growth, and efficiency of feed utilization of Jersey calves to 12 weeks of age

	4:1 Ratio		3:2 Ratio		2:3 Ratio		Av. all calves	
	Control	Aureomycin[a]	Control	Aureomycin[a]	Control	Aureomycin[a]	Control	Aureomycin[a]
No. of calves	4	4	4	4	4	4	4	4
Whole milk *(lb.)*	218	205	214	209	212	221	215	212
Hay *(lb.)*	91.2	99.6	58.2	82.4	68.4	70.6	72.6	84.2
Grain *(lb.)*	20.7	22.4	32.6	48.7	84.9	90.2	46.1	53.8
Birth wt. *(lb.)*	54.5	51.0	53.0	52.5	58.0	54.0	55.0	53.0
Wt. 12 wk. *(lb.)*	104.5	112.0	98.0	120.5	122.0	133.0	107.0	121.0
Wt. gain 12 wk. *(lb.)*	50.0	61.0	45.0	68.0	64.0	79.0	52.0	68.0
Adjusted mean gain 12 wk. *(lb.)*[b]	53.5	66.2	53.5	67.1	54.0	66.0	53.7	66.4
Birth wither ht. *(in.)*	26.4	25.9	25.5	25.8	26.0	26.1	26.0	25.9
Withers ht. 12 wk. *(in.)*	29.4	29.8	28.4	30.1	30.4	30.9	29.3	30.3
Withers ht. gain 12 wk. *(in.)*	3.0	3.9	2.9	4.3	4.4	4.8	3.3	4.4
TDN intake *(lb.)*[c]	96.6	101.0	88.1	111.0	132.6	139.1	106.0	117.0
TDN/lb gain *(lb.)*[c]	1.93	1.66	1.96	1.63	2.07	1.76	2.03	1.71
NE intake *(Therms)*[c]	98.1	100.3	92.9	115.0	138.7	145.6	109.9	120.6
NE/lb gain *(Therms)*[c]	1.96	1.65	2.06	1.69	2.16	1.84	2.11	1.77

[a] 15 mg/day fed in milk 1st 7 wk.; 20 mg/lb of dry feed eaten during the 8th-12th wk.

[b] Mean gain (adjusted for TDN) = Y − bx, where Y = group mean gain, b = regression coefficient and x = deviation from the total mean *(33)*.

[c] TDN and NE calculated from Morrison's *(23)* appendix, Tables 1 and 2.

TABLE 2

The effect of feeding Jersey calves aureomycin for the first 12 weeks of age on the subsequent growth, feed consumption and efficiency of feed utilization

Group during 1st 12 wk.		Control	Aureomycin	Control	Aureomycin
No. on various hay to grain ratios					
1st 12 wk.	4:1	1	1	3	3
	3:2	3	3	0	0
	2:3	1	2	2	2
Hay to grain ratio 12-26 wk.		2:1		4:1	
Total No. of calves 12-26 wk.		5	6	5	5
Hay, 12-26 wk. *(lb.)*		517	531	550	545
Grain, 12-26 wk. *(lb.)*		236	232	136	144
Body wt. 12 wk. *(lb.)*		110	126	107	121
Body wt. 26 wk. *(lb.)*		258	261	231	230
Gain in wt. 12-26 wk. *(lb.)*		148	135	124	109
Adjusted mean gain, 12-26 wk. *(lb.)*[a]		139	126	133	118
Withers ht. 12 wk. *(in.)*		29.3	30.7	29.6	30.0
Withers ht. 26 wk. *(in.)*		35.5	36.1	34.8	35.0
Gain in ht. 12-26 wk. *(in.)*		6.2	5.4	5.2	5.0
TDN intake, 12-26 wk. *(lb.)*[b]		446	450	386	389
TDN/lb gain, 12-26 wk. *(lb.)*[b]		3.00	3.33	3.11	3.57
NE intake, 12-26 wk. *(Therms)*[b]		400	403	337	341
NE/lb gain, 12-26 wk. *(Therms)*[b]		2.70	2.99	2.72	3.13

[a] Mean gain (adjusted for TDN) = Y − bx, where Y = group mean, b = regression coefficient, and x = deviation from total mean *(33)*.

[b] TDN and NE calculated from Morrison's *(23)* appendix, Tables 1 and 2.

compared to that of the calves fed aureomycin during the first 12 weeks and subsequent to the aureomycin feeding period, 12 to 26 weeks of age, are given in Figure 1. It will be noted that the growth advantage at 12 weeks due to

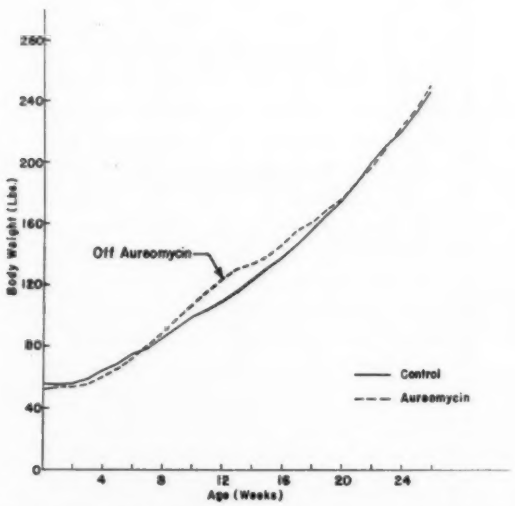

FIG. 1. Changes in body weight during and after aureomycin (Aurofac 2A) feeding to Jersey calves. (15 mg/day first 7 wk. during the milk feeding period and 20 mg/lb of dry feed during the 8th-12th wk.)

aureomycin feeding was nullified within approximately 8 weeks after removing aureomycin from the ration.

It is evident, therefore, from these data that aureomycin feeding resulted in a greater efficiency of feed utilization with increased growth to 12 weeks of age, followed by a period of decreased efficiency of feed utilization and growth after removal of aureomycin from the ration so that little if any advantage existed at 6 months of age.

The calves fed the 2:1 hay to grain ratio during the period of 12 to 26 weeks of age gained more and utilized their feed more efficiently than the calves fed the 4:1 ratio. This was indicated by TDN per pound gain and by the adjusted mean gain (Table 2). Similar findings for efficiency of feed utilization, though not for growth, have been reported previously *(15)*.

The increased efficiency of feed utilization during aureomycin feeding apparently was not due to increased dry matter, cellulose, or protein digestibility, as shown in Table 3. The somewhat greater average amount of nitrogen retained

TABLE 3

Effect of aureomycin feeding on digestion and nitrogen retention in individual 12-week-old Jersey calves

Hay to grain ratios	Dry matter digested		Cellulose digested		Protein digested		N. retained	
	Control	Aureomycin	Control	Aureomycin	Control	Aureomycin	Control	Aureomycin
	(%)	*(%)*	*(%)*	*(%)*	*(%)*	*(%)*	*(g/day)*	*(g/day)*
4:1	67.2	64.5	70.5	68.2	57.5	54.0	8.5	7.8
3:2	68.4	69.4	68.7	69.0	62.0	60.4	9.3	13.4
2:3	68.6	71.8	61.4	59.3	66.8	70.0	13.5	14.6
Av. (3 calves)	68.1	68.6	66.8	65.5	62.1	61.5	10.4	12.0

by the aureomycin-fed calves is of questionable significance because of the small number of calves (Table 3). In both the control and aureomycin groups there was a definite decrease in cellulose digestion when the 2:3 hay to grain ratio was fed, compared to either the 3:2 or the 4:1 ratios. It was also found that the percentage of protein digested and the daily nitrogen retained increased stepwise as the amount of grain fed was increased from the 4:1 hay to grain ratio to the 2:3 ratio. This is in agreement with the observations of Lofgreen *et al. (18)* and others as reviewed by Munro *(21)* that protein digestion increased as the energy in the ration of calves increased.

Riboflavin and thiamine analyses at 12 weeks of age (Table 4) indicated a slight though statistically insignificant difference in the average riboflavin content of both the rumen juice and urine of aureomycin-fed calves, compared to the controls. This is in line with the results of Chance *et al. (11)*. The average thiamine content of both rumen juice and urine, however, was essentially the same in both the aureomycin-fed and control calves. The ratio of hay to grain fed did not appear to markedly influence either the riboflavin or thiamine content of the rumen juice. However, a stepwise decrease in both thiamine and riboflavin content of the urine occurred as the grain feeding was increased from

TABLE 4

Effect of aureomycin feeding on the riboflavin and thiamine content of rumen juice and urine of 12-week-old male Jersey calves

Hay to grain ratio	Rumen juice			
	Riboflavin		Thiamine	
	Control	Aureomycin	Control	Aureomycin
	(γ/100 ml)	*(γ/100 ml)*	*(γ/100 ml)*	*(γ/100 ml)*
4:1	100.0	102.5	14.9	14.0
	140.6	62.5	35.9	17.1
	85.9	153.0	29.8	28.1
	.....	178.0	.....	42.5
3:2	92.3	61.0	38.1	36.0
	156.1	140.5	25.9	16.0
	.....	117.2	.....	26.5
				43.0
2:3	132.8	93.8	29.8	27.9
	140.6	145.3	13.2	34.8
	101.5	65.7	34.7	33.0
	109.5	.....	20.0	.....
Av.	121.4	107.5	26.9	29.0
	Urine			
	Riboflavin		Thiamine	
	(mg/24 hr/100 lb)		*(mg/24 hr/100 lb)*	
4:1	1.56	1.47	3.26	2.18
3:2	2.42	1.10	1.43	1.94
2:3	2.14	0.92	1.27	1.46
Av.	2.04	1.16	1.99	1.86

the 4:1 hay to grain ratio to the 2:3 ratio, with the exception of the low value obtained for riboflavin in the 4:1 ratio, control calves (Table 4). The significance of these changes cannot be readily explained.

It was of particular interest that the aureomycin-fed calves maintained a significantly higher ($P < 0.01$) blood sugar level during the last 4 weeks of the aureomycin feeding period than did the controls (Figure 2). Both groups declined rapidly during the first 8 weeks from a relatively high level, 80-90 mg.%, to a lower level, 50-60 mg.%. This decline in blood sugar level with increasing age is believed by McCandless and Dye *(20)* to indicate the transition period from the simple-stomached animal to the ruminant. However, Reid *(29)*, working with lambs, found that the major portion of the decrease in blood glucose during the first 4 or 5 weeks was in corpuscle glucose and that plasma glucose later fell to the adult level. His conclusion was that the changes in blood glucose were not associated with functional development of the rumen.

Chromatographic separation of acetic, propionic, and butyric acids in the rumen juice at 12 weeks of age indicated that there was a shift in the average proportion of propionic acid and butyric acid due to aureomycin feeding (Table 5). Although the average per cent of acetic acid was approximately the same in both groups, there was a lower per cent of propionic acid and a higher per

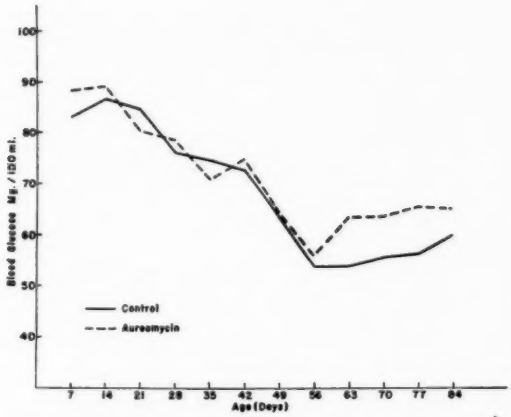

FIG. 2. Changes in the blood glucose level of Jersey calves raised on the high roughage system with and without aureomycin supplement. (15 mg/day first 7 wk. during the milk feeding period and 20 mg/lb of dry feed during the 8th-12th wk.)

cent of butyric acid in the rumen juice of the aureomycin-fed calves than in that of the controls. The total of the three acids was approximately the same for each group. As the grain increased in proportion to the hay fed, a definite shift in proportion of propionic and butyric acids was observed. The propionic acid content decreased and the butyric acid increased. Acetic acid was found to be somewhat lower in the group fed the 2:3 ratio of hay to grain (Table 5). No significance could be attached to the small differences observed in the total steam volatile fatty acids. Inasmuch as the fatty acids measured in rumen juice represent only the acids which are not yet absorbed, these changes may reflect much greater differences in actual production of the various fatty acids in the rumen.

The question is raised as to whether the lowered propionic acid and the higher butyric acid content in the rumen juice of the aureomycin fed calves may be associated with the higher blood sugar of these calves. Further, it might be questioned whether or not the shift in propionic and butyric acids in the rumen juice and the increased blood sugar may be associated with the increased growth and efficiency of feed utilization of the aureomycin-fed calves. If so, the mode of action of aureomycin as a growth stimulant may be closely associated with energy metabolism, possibly an energy sparing effect elicited through its effect on the microflora of the rumen and lower intestinal tract.

That at least a part of the effect of aureomycin was elicited through the rumen bacteria is indicated by the effect of aureomycin feeding on the average ratings of various indicator rumen microorganisms (Table 6). It will be noted that neither the protozoa rating nor rating for the Hay I group bacteria was affected by aureomycin feeding. On the other hand, the Hay II group bacteria failed to become well established in the rumens of the aureomycin-fed calves, whereas

TABLE 5

The effect of aureomycin feeding on the volatile fatty acid content of rumen juice of 12-week-old Jersey calves

	4:1 Ratio		3:2 Ratio		2:3 Ratio		Av. all calves	
	Control	Aureomycin	Control	Aureomycin	Control	Aureomycin	Control	Aureomycin
No. of calves	4	2	4	4	2	3	10	9
Total steam volatile fatty acids *(Meq/l)*	88.0	86.0	84.8	80.0	84.5	82.0	85.9	82.0
Av. acetic: *(g/l)*	3.16	3.64	3.44	3.01	2.36	2.61	3.11	3.02
(%)	72.5	69.6	66.5	70.6	63.6	64.7	68.5	68.5
Av. propionic: *(g/l)*	0.90	1.11	1.09	0.72	0.68	0.58	0.93	0.76
(%)	20.6	21.1	21.1	17.2	18.3	14.3	20.3	17.2
Av. butyric: *(g/l)*	0.30	0.49	0.64	0.55	0.67	0.85	0.51	0.63
(%)	6.9	9.3	12.4	12.2	18.1	21.0	11.2	14.3
Av. total: *(g/l)*[a]	4.36	5.24	5.17	4.28	3.71	4.04	4.55	4.41

[a] Total acetic, propionic, and butyric acid.

TABLE 6

Effect of aureomycin feeding on the average rating of rumen indicator microorganisms in Jersey calves

Age range	Indicator microorganisms rated[a]	4:1 Ratio		3:2 Ratio		2:3 Ratio	
		Control	Aureomycin	Control	Aureomycin	Control	Aureomycin
(wk.)							
4-5	Protozoa[b]	1.7 (3)[e]	2.2 (4)	1.0 (3)	2.8 (4)	2.3 (4)	1.0 (4)
	Hay I[c]	2.7 (3)	2.7 (3)	2.3 (3)	1.8 (4)	3.8 (4)	1.5 (4)
	Hay II[d]	2.0 (3)	1.0 (3)	1.0 (3)	1.0 (4)	1.3 (4)	0.0 (4)
6-7	Protozoa	2.0 (4)	3.3 (3)	3.0 (3)	3.3 (4)	2.7 (3)	3.0 (4)
	Hay I	2.0 (2)	2.3 (3)	3.5 (2)	3.5 (4)	2.7 (3)	1.3 (4)
	Hay II	3.0 (2)	1.3 (2)	3.0 (2)	0.5 (4)	1.0 (3)	0.3 (4)
9-10	Protozoa	3.3 (3)	4.0 (2)	3.3 (4)	4.0 (3)	3.0 (3)	2.7 (3)
	Hay I	2.3 (4)	3.7 (3)	2.5 (4)	3.5 (4)	3.3 (4)	3.8 (4)
	Hay II	6.0 (4)	1.0 (3)	3.3 (4)	1.5 (4)	2.0 (4)	0.5 (4)
11-12	Protozoa	4.0 (4)	3.8 (4)	3.7 (3)	3.8 (3)	4.0 (3)	4.0 (4)
	Hay I	2.5 (2)	3.2 (4)	3.0 (4)	3.7 (3)	2.3 (3)	2.8 (4)
	Hay II	6.6 (3)	0.5 (4)	4.5 (4)	1.3 (3)	0.7 (3)	0.0 (4)

[a] Indicator microorganisms and method as previously described *(27)*.
[b] Protozoa rated 0-4 using wet preparations.
[c] Hay I — one bacterium rated 0-4, using Gram's stain.
[d] Hay II — three bacteria individually rated 0-4 and summed, using Gram's stain.
[e] Figures in parentheses indicate number of calves represented in the average.

they became readily established in the control calves. It is also noteworthy that the ratings for the Hay II group bacteria were greatly reduced as the grain feeding was increased from the 4:1 hay to grain ratio to the 2:3 ratio. The increased grain feeding did not materially influence the ratings of either the protozoa or the bacteria in the Hay I group.

Neumann *(26)* observed no difference in total numbers of rumen microorganisms but reported the absence of certain varieties of microorganisms in aureomycin-fed cattle. The changes in the rumen microorganisms are particularly significant in the light of the results of McGilliard *et al. (21)*, who showed that calves given rumen inoculations after the termination of aureomycin feeding gained faster than calves which had been aureomycin-fed but were uninoculated.

The decreased growth and efficiency of feed utilization after termination of aureomycin feeding in our experiment may have been associated with the absence of types of microorganisms that are represented by indicator bacteria from the Hay II group.

Bacteria from the Hay II group have been shown, both in the data presented in Table 6 and in a previous publication *(28)*, to be poorly established when a high proportion of grain to hay is fed. This may account for the fact that Rusoff *et al. (32)*, using the indicator method *(27)*, found no differences in rumen microorganisms that could be attributed to aureomycin when calves were fed a ration consisting largely of a calf starter.

Although aureomycin feeding increased growth and efficiency of feed utilization during the period of administration, this advantage would be economically unimportant so far as dairy herd replacements are concerned because of its nullification after aureomycin feeding was terminated. Any advantages due to improved health were not apparent in this study.

The finding that digestion of dry matter, cellulose, and protein was unaffected by aureomycin feeding shows that the increased growth resulted from some cause other than increased digestibility of the feed. The primary cause of increased growth due to aureomycin feeding, though involving the microflora of the digestive tract, may be closely associated with energy utilization and metabolism, possibly the result of a change in the form or metabolic pathways of ingested energy sources. This is indicated by the higher level of blood sugar, and the shift observed in the relative proportion of propionic and butyric acids in the rumen juice.

SUMMARY

Twenty-four Jersey calves were used in a study of the effects of feeding aureomycin to calves raised on the high roughage system using three ratios of hay to grain (4:1, 3:2, and 2:3). Aureomycin in the form of Aurofac 2A was fed at the rate of 15 mg. per day for the first 7 weeks during milk feeding and at the rate of 20 mg. per pound of dry feed ingested from the end of the seventh week to 12 weeks of age.

Aureomycin increased growth both in body weight and withers height and resulted in increased feed intake and efficiency of feed utilization at all three hay to grain ratios. After the termination of aureomycin feeding, both growth and efficiency of feed utilization declined below the level of the controls so that no prolonged growth benefit resulted at either hay to grain ratio fed, 2:1 or 4:1. The percentage of dry matter, cellulose, and protein digested was not influenced by aureomycin feeding.

When the grain was increased from the 4:1 to the 2:3 hay to grain ratio, growth increased in accordance with the increased TDN intake, cellulose digestion decreased, and protein digestion and nitrogen retention increased.

Riboflavin appeared to be higher in both rumen juice and urine when aureomycin was fed. Thiamine was not affected by aureomycin in either rumen juice or urine.

Although the total volatile fatty acid and acetic acid content of rumen juice was not changed by aureomycin feeding, a shift occurred in the relative proportion of propionic and butyric acids. In the aureomycin-fed group, the average butyric acid content of the rumen juice was higher and propionic acid was lower than in that of the control group. The stepwise increase in the proportion of hay to grain fed resulted in lower per cent acetic and propionic acids and increased per cent butyric acid.

During the period 8 to 12 weeks, blood sugar was maintained at a higher level in the aureomycin-fed group than in the control group.

Ratings of indicator microorganisms, made from wet preparations and Gram stains, showed that aureomycin feeding practically eliminated the establishment of the Hay II group of rumen bacteria, which were well established in the control group. Increasing the amount of grain fed in relation to hay resulted in a progressive decrease in rumen bacteria of the Hay II group, which is in agreement with previously published results.

The evidence obtained suggests that the growth stimulation due to aureomycin feeding resulted from an alteration in energy metabolism, possibly involving the microflora of the rumen.

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IN VIVO (DAIRY CALVES) AND IN VITRO STUDIES WITH SURFACE-ACTIVE AGENTS[1,2]

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Recently it has been suggested that various surface-active agents when administered in the diet may effect growth responses similar to those observed with antibiotics. The reports have not been entirely consistent, however, for in some studies detergents have been found to stimulate growth in chicks *(6, 7, 8)* and in pigs *(3, 5, 11)*, whereas in others no effects on growth in these species were observed *(13, 14, 16)*. Since the same detergents were not used in all of the studies, the observed differences may have been due to this variation.

Information relative to the effects of surface-active agents on growth of dairy calves is not extensive. Lassiter *et al. (10)* reported growth stimulation from inclusion of Ethomid C/15-16 in the diets of calves, whereas Gardner *et al. (9)* found that Ethomid C/15 had no effect on calf growth. Therefore, further study on the comparative effects of detergents on growth of calves was indicated. Moreover, since it has been proposed that detergents and antibiotics may exert their growth-stimulating effect, at least in part, through reduction of surface tension *(2)*, investigation of this suggested relationship seemed desirable.

EXPERIMENTAL

The studies reported herein were divided into two phases. The first phase (Experiment I) was conducted to determine the effects of various detergents upon growth, physical well-being, and certain blood components of dairy calves. The second phase (Experiment II) was designed to determine the comparative effects of several detergents and antibiotics on the surface tension of distilled water and bovine rumen fluid.

EXPERIMENT I

To determine the effect of detergents on calves of various ages, Experiment I was subdivided further so that part of the calves were placed on experiment at 4 days of age (Trial 1) and the others at an average age of 13 weeks (Trial 2).

TRIAL 1.

Procedure. Sixteen Holstein calves, eight males and eight females, were permitted to remain with their respective dams for 3 days postpartum. Subsequently, they were transferred to individual pens, and the calves of each sex were allotted randomly to two groups. Whole milk averaging 3.0% fat was

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fed to the calves at the daily rate of 5.0 and 3.6 lb. per 100 lb. body weight during the first and second weeks, respectively, of the experiment. A milk replacement containing primarily dried whey product reconstituted with water (14% whey product, 86% water) was fed the first 7 weeks at the following daily rates per 100 lb. body weight: 3.0, 5.4, 10, 8, 8, 6, and 4 lb., respectively. A calf starter[3] was fed ad libitum to a maximum of 4 lb. daily per calf, and medium quality alfalfa-brome grass hay was fed free choice. One group of calves was fed a cationic detergent (Aerosol C-61) in the milk replacement at the daily rate of 1 g. per calf during the initial 7 weeks. From 7 to 12 weeks this detergent was fed in the calf starter at the daily rate of 2 g. per calf.

Feed consumption was recorded daily; body weight was recorded weekly; and height at withers, chest circumference, and barrel circumference were recorded at 4-week intervals. Incidence of diarrhea was estimated by daily observations of fecal samples obtained by manual stimulation of defecation. Fecal pH values were determined regularly each week and daily during periods of severe diarrhea. Samples of venous blood were obtained at weekly intervals; hematological observations included plasma fat *(1)*, hemoglobin *(17)*, and erythrocyte, total leucocyte, and differential leucocyte counts *(17)*.

TABLE 1

Mean body measurements and plasma fat levels of calves in Trial 1 (Experiment 1)

Measurement	Group[a]	Weeks on experiment							Total increase
		0	2	4	6	8	10	12	
Body wt.	C	93	94	98	112	127	144	162	69
(lb.)	D	90	87	94	106	117	134	153	63
Ht. at withers	C	29.2		29.6		30.8		32.1	2.9
(in.)	D	28.9		29.6		30.5		31.7	2.8
Chest circ.	C	31.5		34.3		36.1		38.4	6.9
(in.)	D	31.3		32.8		34.8		36.8	5.5
Barrel circ.	C	32.2		36.9		42.3		47.8	15.6
(in.)	D	32.3		37.1		42.0		45.2	12.9
Plasma fat	C	137	96	59	49	80	102	122	
(mg/100ml)	D	152	112	66	42	70	94	115	

[a] Eight Holstein calves per group; C = control; D = detergent (Aerosol C-61).

Results. Mean body measurements and plasma fat values are summarized in Table 1. Analysis of variance *(15)* of these data indicated no differences between groups during the 12-week period. The mean total feed consumption per calf for the control and detergent groups, respectively, was: hay, 72 and 66 lb.; starter, 147 and 146 lb. There were no marked differences between groups in trends of weekly feed consumption.

No appreciable differences were apparent between groups in incidence or severity of diarrhea. The highest incidence of scouring was during the period from 3 to 5 weeks and was coincident with the highest levels of milk replace-

[3] Composed of 40% ground corn, 30% ground oats, 28% soybean oil meal, 1% steamed bone meal, and 1% iodized salt.

ment feeding. Fecal pH values were extremely variable and apparently were not influenced greatly by diarrhea. No consistent differences in pH were noted between groups; the control group averaged 6.59 and the detergent group 6.71.

Neither the plasma levels of fat (Table 1) nor the appearance of the plasma was influenced by feeding Aerosol C-61. Also, no differences between groups in hemoglobin, erythrocytes, total leucocytes, lymphocytes, neutrophiles, monocytes, eosinophiles, or basophiles were observed.

TRIAL 2.

Procedure. Twenty-four calves (12 Holsteins, eight Brown Swiss, and four Guernseys) were divided into two groups on the basis of age, breed, sex, and nature of previous diet. The calves averaged 13 weeks of age when started on experiment. A concentrate mixture[4] was fed ad libitum to a maximum of 6 lb. daily per calf, and medium quality mixed hay, mostly alfalfa and brome grass, was fed free choice. A limited amount of whole milk was fed for the first 3 weeks to eight calves (four youngest pairs) that were receiving whole milk when assigned to this experiment. Aerosol SE, Aerosol AN, and TEF-16, representing respectively cationic, anionic, and nonionic types of surface-active agents, were fed for an 11-week period. The detergent Tide also was included in the study. Each surface-active agent was fed to 2-4 calves at the rate of 1 lb. per 1,000 lb. of the concentrate feed mixture.

Feed consumption was recorded daily; body weight was recorded weekly; and height at withers, chest circumference, and barrel circumference were recorded at 4-week intervals and at the termination of the trial. Observations were made daily for diarrhea and for general well-being of the calves. At approximately 2-week intervals, samples of venous blood were obtained (4-5 hours after concentrate and detergent feeding) for determination of plasma fat *(1)*.

Results. Preliminary observations indicated that concentrate mixtures containing certain detergents were comparatively unpalatable. The calves refused to eat feed containing Aerosol OT; therefore, this detergent could not be used. Tide fed at levels in excess of 1 lb. per 1,000 lb. concentrates resulted in feed refusals. However, the surface-active agents selected for the 11-week feeding trial apparently were acceptable to the calves at the levels employed.

No major differences in growth response to the various detergents were apparent, although the small number of calves assigned to each detergent limited the reliability of such comparisons. It was decided, therefore, to combine the data for all calves fed the various surface-active agents to permit comparison with the averages for the control calves. The mean values (Table 2) indicate that detergent feeding had no appreciable effect on growth or on blood plasma fat levels. Moreover, feed consumption values of the two groups were similar. The mean totals per calf for the control and for the detergent groups,

[4] Composed of 30% ground corn, 15% ground oats, 15% wheat bran, 8% dried beet pulp, 5% dried skimmilk, 12% linseed oil meal, 12% soybean oil meal, 2% steamed bone meal, and 1% iodized salt.

TABLE 2

Mean body measurements and plasma fat levels of calves in Trial 2 (Experiment 1)

Measurement	Group[a]	Weeks on experiment						Total increase
		0	2	4	6	8	11	
Body wt.	C	182	202	223	238	264	305	123
(lb.)	D	187	203	218	237	261	303	116
Ht. at withers	C	33.4		35.2		36.6	37.3	3.9
(in.)	D	33.7		35.1		36.7	37.3	3.6
Chest circ.	C	38.7		41.8		44.8	45.6	6.9
(in.)	D	39.4		41.4		43.7	45.5	6.1
Barrel circ.	C	45.6		50.6		55.0	57.5	11.9
(in.)	D	47.4		50.4		55.1	57.3	9.9
Plasma fat	C		103	119	120	119	138	
(mg/100 ml)	D		112	129	126	124	125	

[a] Twelve calves per group (each composed of 4 Brown Swiss, 2 Guernseys, and 6 Holsteins); C = control; D = detergent.

respectively, were: hay, 249 and 249 lb.; starter, 390 and 378 lb. Incidence of diarrhea was somewhat greater among the detergent-fed calves, a difference which was due primarily to frequent scouring of two calves fed Tide and one calf fed Aerosol SE.

EXPERIMENT II

The in vitro effects of a surface-active agent (Dural H[5]) and of several antibiotics on the surface tension of distilled water were determined at concentrations ranging from 25 to 200 mg. per 100 ml. Surface-tension measurements, a minimum of three per sample, were made with a du Noüy tensiometer. The mean values, presented in Figure 1, indicate that the surface tension of distilled water was markedly reduced by Dural H, even at comparatively low concentrations; the hydrochlorides of aureomycin and terramycin had little effect; and procaine penicillin G was intermediate. Aureomycin and terramycin were not completely soluble at concentrations above 50 mg. per 100 ml. of distilled water; therefore, the significance of these surface-tension curves at higher concentrations should be minimized.

It seemed desirable, further, to investigate the effects of antibiotics and detergents on the surface tension of gastrointestinal ingesta, and, because of accessibility, rumen ingesta were selected for study. A sample of rumen contents from a Holstein cow (with a rumen fistula) was obtained and was filtered through cheesecloth to obtain the rumen fluid. The surface tension of an aliquot of this fluid was determined (du Noüy tensiometer) initially and several times during the experiment. No major changes with time were apparent in the untreated fluid. Surface-active agents and antibiotics (in crystalline form) were added to other aliquots of the rumen fluid at levels of 0.5, 1, 2.5, and 5 mg. per 100 ml., and surface tension values were determined. The results at the 5-mg. level, which gave the greatest response (Table 3), indicate that the detergents and penicillin markedly reduced the surface tension, whereas aureomycin

[5] Obtained from Wyandotte Chemical Co., Wyandotte, Michigan.

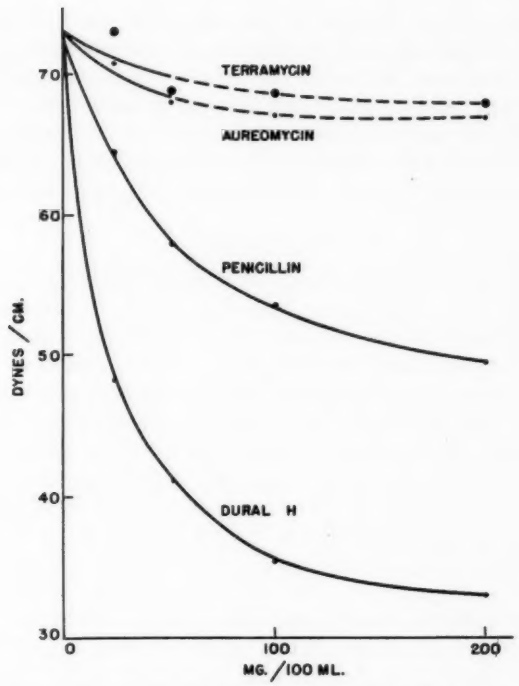

FIG. 1. Effects of several antibiotics and a detergent (Dural H) on the surface tension of distilled water.

and terramycin had very little effect. These comparative changes were similar to those obtained with distilled water.

Three animals with rumen fistulas were employed to study the effects of aureomycin on surface tension of rumen fluid in vivo. One of two yearling Milking Shorthorn males received the basal ration (alfalfa hay and concen-

TABLE 3

Effect of surface-active agents and antibiotics on surface tension of rumen fluid

Additions to rumen fluid[a]	Surface tension
	(dynes/cm)
None	56.58
Aerosol OS (anionic)	48.2
Aerosol AN (anionic)	42.2
Aerosol SE (cationic)	51.9
Tide	48.6
Aureomycin hydrochloride	55.2
Terramycin hydrochloride	54.9
Procaine penicillin G.	48.6

[a]At rate of 5 mg. per 100 ml.

trates,[6] approximate ratio of 2 to 1) and the other was fed the basal ration plus 240 mg. of aureomycin daily. At the end of 2 weeks the diets were reversed. A third animal (a mature Holstein cow) received the basal ration continuously. A sample of rumen fluid was collected daily from each animal; the fluid then was filtered, and the surface tension was measured as described earlier. The surface tension of the fresh rumen fluid (no aureomycin) averaged 52 dynes per centimeter, and the feeding of aureomycin did not effect any significant changes.

DISCUSSION

In the present investigation the feeding of detergents to dairy calves had no significant effect on growth, as evaluated by body weight changes and various other body measurements, on incidence of diarrhea or on physical appearance. Only a limited number of detergents (anionic, cationic, and nonionic) were included. Thus, there is a possibility that marked variations exist in the effects of various detergents and that the results observed herein are not necessarily representative of the effects of other detergents. Moreover, the detergents may not have been included in amounts sufficient for optimum growth stimulation, although the level fed in the concentrate mixture (1:1,000) was similar to those employed elsewhere for dairy calves *(9, 10)* and for other species *(8, 11)*.

Chung and Shaw *(4)* have observed a sustained elevation of blood plasma lipids and a frothy appearance of the blood plasma after intravenous injection in goats and in a lactating cow of a detergent (Triton WR 1339). In the studies reported herein, the oral administration of the various detergents had no apparent influence on the physical appearance of the blood plasma or on the plasma fat levels. This is in agreement with the results of Perry *et al. (12)*, who reported that the feeding of surface-active agents at a level of 0.26% of the diet of pigs failed to alter the blood plasma lipid values.

In the present investigation it has been demonstrated that aureomycin and terramycin, which have been shown to promote growth in the young dairy calf, effect only minor changes in the surface tension of distilled water and of rumen fluid. On the other hand, penicillin, which is less effective as a growth stimulant in the calf, causes an appreciable reduction in the surface tension of the above liquids. Moreover, the surface-active agents evaluated in the present in vitro study markedly reduced surface tension, whereas those that were fed failed to increase rate of growth. It would seem, therefore, that the relative growth-stimulating action of some of the antibiotics is not effected primarily through reduction in surface tension. It is possible, however, that the effects on distilled water and on rumen fluid observed herein may not have been typical of changes produced in the lower portion of the gastrointestinal tract.

SUMMARY

The feeding of several surface-active agents had no significant influence on the growth rates of dairy calves when fed either in a milk replacement diet or

[6] Composed of 27.5% ground corn, 34.5% ground oats, 14% wheat bran, 7% linseed oil meal, 14% soybean oil meal, 2% steamed bone meal, and 1% iodized salt.

in a concentrate mixture. Moreover, the detergents had no consistent effect on hay and concentrate consumption, incidence of diarrhea, fecal pH, blood plasma fat levels, or on hemoglobin, erythrocyte, leucocyte and differential leucocyte values of the blood. The addition of surface-active agents and of penicillin to distilled water and to rumen fluid caused relatively large reductions in surface tension, whereas aureomycin and terramycin had no appreciable influence on the surface tension of these liquids.

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A SELECTION INDEX FOR FAT PRODUCTION IN DAIRY CATTLE UTILIZING THE FAT YIELDS OF THE COW AND HER CLOSE RELATIVES [1]

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Improvement of traits with low heritability can often be enhanced if, in the selections, appropriate attention is paid to the phenotypes of close relatives in addition to the animal's own phenotype. When selection is directed toward improving a single trait, the importance of the individual's own phenotype, as compared with the phenotype of a particular relative, for predicting an animal's breeding value is determined by the heritability of the trait and the closeness of the relative's genetic relationship. The individual's phenotype receives nearly all the emphasis when heritability is high, but there is room for more attention to the relative's phenotype when heritability is low.

Lush *(3)* explored the relative worth of the individual's performance alone and the performance of progeny alone for predicting individual breeding values. Information on at least five progeny was needed to equal the information provided by the individual's phenotype, unless some selection had already been practiced and only the differences among the individuals surviving were being considered. Lush *(5)* also investigated the results anticipated from selecting on individuality alone, solely on the family average, or on the optimum combination of the two. Only if the phenotypic correlation between family members and the genetic correlation between family members are distinctly unequal, does the family provide information which makes selection much more accurate than if selection were based on the individual's own performance alone.

The present study was undertaken to derive an index for more accurate intraherd selection for fat production by utilizing properly all of the information on the individual cow and her close relatives. Fat yields of the cow, her dam, her daughters, her maternal sisters, and her paternal sisters are used to predict the cow's breeding value. The proposed index combines these items of information about the cow and these relatives in such a way that the index value for the cow will be correlated more closely with her breeding value than if the information were combined in any other linear manner.

EXPERIMENTAL

Data for this study were obtained from the American Jersey Cattle Club. The data included fat production records of all herds on Herd Improvement Registry Test for at least 4 years during the period from 1943 to 1947. In all,

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23,330 lactation records for 12,405 cows in 293 herds were studied. These herds were located in 42 states.

All available completed lactations, which were not less than 270 days in length, for all cows in the sampled herds were used. Where the lactation was more than 305 days in length, only the fat production during the first 305 days was used. The records were brought to a mature equivalent, twice-a-day milking basis by the American Jersey Cattle Club. The mean fat production on this basis was 429 lb. The variation in the general mean from year to year was small, causing barely more than half of one per cent of the variance between individual records. The records completed in the calendar years 1943 to 1947 averaged 421, 420, 433, 432, and 439, respectively.

The statistics required to construct the index were obtained by analyzing the data just described. Although previous estimates of some of these statistics were available from the literature, analysis of a sizable volume of unselected data was desired in order to provide statistics with negligible sampling errors and general applicability. The statistics required from the data were:

1. Repeatability of fat records of the same cow.
2. Correlation between fat records of maternal half sisters.
3. Correlation between fat records of paternal half sisters.
4. Heritability of fat production.

These basic statistics and the path coefficient approach to the biometrical relations among relatives *(10)* were employed to construct the index. The first three statistics were computed as intraclass correlations, by separating the observed variance into its components. Heritability was computed by doubling the intraherd regression of daughter on dam.

RESULTS AND DISCUSSION

Repeatability. The analysis of variance used to obtain the components of variance between herds, between years within herd, between cows within herd, and within cows, is summarized in Table 1. Values for σ^2, σ^2y, and σ^2e were obtained by solving any three of the last four lines, which are equations for the

TABLE 1

Analysis of variance for the influence of herd, year within herd, and cow

Source of variation	Degrees of freedom	Mean square	Expected composition of mean square
Total	23,329	10,498	
Among herds	292	342,207	σ^2 [a] + 2.342 σ^2e [b] + 19.177 σ^2y [c] + 79.482 σ^2h [d]
Intraherd variance:			
Years	1,062	13,527	$\sigma^2 + 0.630\ \sigma^2e + 16.669\ \sigma^2y$
Within years	21,975	5,944	$\sigma^2 + \sigma^2e$
Cows	12,112	8,607	$\sigma^2 + 1.870\ \sigma^2e + 0.560\ \sigma^2y$
Within cows	10,925	3,729	$\sigma^2 + \sigma^2y$

[a] σ^2 is variance among records of the same cow, adjusted for year effect.
[b] σ^2e is the additional variance among records of different cows.
[c] σ^2y is variance due to year-to-year variation within herds.
[d] σ^2h is variance due to genetic and environmental differences among herds.

TABLE 2

Components of variance as fractions of the total and of the intraherd variance

Component	Value	Fraction of total variance	Fraction of intraherd variance
σ^2h	4060	0.386	.....
σ^2y	515	0.049	0.080
σ^2c	2730	0.259	0.422
σ^2	3214	0.306	0.498

mean squares: years in herds, within years, cows in herds, and within cows. The values for these components were substituted into the equation for the mean square among herds to solve for σ^2h.

Components derived from the analysis summarized in Table 1 are presented in Table 2 in absolute numbers and also as fractions of the total and of the intraherd variances. The principal statistic needed from these components was the repeatability. Among records appropriately adjusted for year-to-year environmental influences, the intraherd repeatability $\left(\frac{\sigma^2c}{\sigma^2c + \sigma^2}\right)$ is 0.459. This value cannot be used in practice unless lactation records can be adjusted accurately for yearly environmental changes. In the usual case, where such adjustment for yearly changes is not made, repeatability can be computed from the last two lines of Table 1. The component σ^2y is then ignored in the mean square expectations and the new values obtained for σ^2c and σ^2 (the definitions of which were thus changed slightly) are 2,609 and 3,729, respectively. These give 0.412, with a probability of 0.9 that the true value really is not less than 0.399 nor more than 0.432, as the repeatability of fat production where no adjustment is made for yearly influences. Year-to-year variations which affect all cows in the herd alike accounted for 8% of the intraherd variance in these data, and Plum *(8)* found they accounted for slightly more than 10% of the intraherd variance. That year-to-year variation was more important within herds than in the general population merely shows that most of the things which make a herd average rise or fall from one year to another affect each herd independently and thus cancel each other's effects in a national average of many herds.

The herd component comprises 39% of the total variance. This is somewhat larger than the fraction reported by Plum *(8)* and by Lush and Straus *(7)*. They found that herd differences accounted for 35 and 30%, respectively, of the total variance. The proportionately larger value in the present study might reflect the additional contribution from wider climatic differences, since the other studies cited were of data from herds in only one state. No way to analyze the herd component further was found. Subsequent statements pertain to differences within herds.

Paternal half sister and maternal half sister correlations. Since paternal half sisters have a common sire, the variance within groups of paternal half sisters is reduced by an amount due to differences in transmitting ability among the sires. The analysis to determine the intraclass correlation between paternal half sisters (full sisters were excluded) separates the variance due to cow differ-

ences into variance among sires, σ^2s, and that among cows with a common sire, σ^2p. Progeny of 4,334 sires were included in the data. The component of variance, σ^2s, was 741 and σ^2p was 2090. The intraclass correlation between paternal half sisters all kept in the same herd with records adjusted for year-to-year effects is 0.123 (i.e., $\frac{\sigma^2s}{\sigma^2s + \sigma^2p + \sigma^2} = 0.123$) where the value for σ^2 is that in Table 2. If yearly influences are ignored, σ^2 and σ^2s increase and σ^2p decreases but the correlation changes only slightly, becoming 0.120.

The intraclass correlation between maternal sisters is needed for knowing the proper weight to put on information on a cow and on her daughters. The variation due to differences between cows was separated into that caused by the cow's dam, σ^2d, and that among cows with a common dam, σ^2m. The component σ^2d was 455 and the component σ^2m was 2,282. The correlation between maternal half sisters kept in the same herd, with their records appropriately adjusted for year-to-year influences, was 0.077 (i.e., $\frac{\sigma^2d}{\sigma^2d + \sigma^2m + \sigma^2} = 0.077$). If the year-to-year influences are ignored the correlation becomes 0.073. Among the 9,914 dams included in the data, 8,013 had only one daughter. Many of the dams were mated to the same sire, and many of the comparisons among the progeny of different dams involved contrasts between paternal half sisters. This makes the mean square among dams and σ^2d smaller than if several sires had been used at one time and may explain why the correlation was smaller among maternal half sibs than among paternal half sibs. This circumstance is not singularly characteristic of these data, but it is to be found wherever only one or two sires are in service concurrently.

The values obtained in this present study agree closely with those reported by Johansson and Hansson *(2)*. They found the intraherd correlation between paternal half sisters to be 0.12 for fat yields corrected for age and length of calving interval. The intraherd correlation for maternal half sisters was 0.06.

Heritability. The intrasire regression of daughter on dam has been a most convenient procedure for estimating the heritability of traits in dairy cattle *(6)*. Most of the herd differences, presumably largely environmental, are removed in the differences among sires. Complete removal results, however, only if all the sire's daughters and mates are born and kept in the same herd. Where data are collected over a long period of time, trends in management may contribute much to the resemblance between daughter and dam, even on an intraherd basis, but not much when computed on an intrasire basis. The progeny of a sire are usually contemporaries. Most of the variance attributable to managemental trends would go into the differences among sires where the analysis is done on an intrasire basis.

For the present data, the herds in which both the daughters and their dams made their records could be identified. Likewise, these data were collected within 5 years, during which there was not an important general time trend within the whole body of data. Thus, the intraherd rather than the intrasire regression was

computed. The average of all available records was used, both for the daughter and for the dam, and the resultant regression was adjusted to the basis of a single record, using the formula presented by Lush and Straus *(7)*.

The total data available were 3,363 dams having 4,764 daughters. The analysis of variance for the dams is in Table 3 and the analysis of covariance between them and their daughters is in Table 4. The number of daughters per dam

TABLE 3

Analysis of variance of dams' fat production

Source of variation	Degrees of freedom	Mean square	Expectation of mean square	Components
Total	3,362	9,067		$\sigma^2y = 515$[a]
Among herds	289	51,253	$\sigma^2e + 3.605\ \sigma^2y + 11.572\ \sigma^2h$	$\sigma^2h = 3,840$
Within herds	3,073	5,100	$\sigma^2e + 0.286\ \sigma^2y$	$\sigma^2e = 4,952$

[a] from Table 2

TABLE 4

Analysis of covariance for daughters and dams

Source of covariance	Degrees of freedom	Mean product	Expectation of mean product	Components
Total	4,762.2	4,721		$\sigma^2y = 515$
Among herds	481.4	40,451	$\frac{\sigma^2g}{2} + 1.847\ \sigma^2y + 9.839\ \sigma^2h$	$\sigma^2h = 3,944$
Within herds	4,280.8	703	$\frac{\sigma^2g}{2} + 0.010\ \sigma^2y$	$\frac{\sigma^2g}{2} = 698$

varied from one to six with a mean of 1.4. Although including all daughters does furnish added information, the total number of degrees of freedom for estimating the intraherd covariance is somewhat less than the total number of daughters minus the number of herds. The degrees of freedom in Table 4 are fractional since they indicate the expected number of $\frac{\sigma^2g}{2}$ components in the cross products as a consequence of some of the dams having more than one daughter in the same herd.

On an intraherd basis where the influence of year-to-year changes in environment are considered, the regression of daughter on dam was 698/4952 or 0.141. Ignoring the variance due to year effects, the intraherd regression is 703/5100 = 0.138. The difference is small because using the average of all records for daughters and for dams cancelled most of the confusing influence of yearly environmental changes.

After the intraherd regressions were adjusted to a single record basis and multiplied by two, the estimate of the heritability of differences in single lactation records adjusted for year effects was 0.212 ± 0.025. When the variance due to year effects was not considered, the estimate was 0.201. These results are bracketed by the value of 0.17 obtained by Lush and Straus *(7)* and 0.28 reported by Tyler and Hyatt *(9)*.

The index. Lush *(5)* has indicated the general area where relatives add

sufficient information to make them worth considering in predicting an individual's breeding value. The phenotypic correlation *(t)* and the genetic correlation *(r)* between family members must be widely different if attention to the family average is to increase the accuracy of selections much. In the present data on an intraherd basis t is 0.12 for paternal half sisters and 0.07 for maternal half sisters. Hence, little is to be gained if r is less than 0.25. Even when r is 0.25 to 0.30, the gain from information on relatives is only moderate, unless the number in each family is large. Consequently, only daughter-dam and half-sister relationships were considered in choosing relatives worth using in this study. (In practice a full sister gets nearly proper weight by being included twice, once as a paternal sister and again as a maternal sister.)

For traits which may be expressed several times during an animal's lifetime, the phenotypes of the several individuals in the herd will be known with different degrees of accuracy, depending largely upon the number of records each animal has completed. An index for repeatable traits, such as milk and fat production, should take into account this variation in the accuracy with which the individual phenotypes are known. In addition, the variation in the number of daughters and maternal and paternal sisters must be considered.

All possible simple correlations between the cow's genotype and phenotype for fat production and the genotype and phenotype for fat production of her dam, her daughters, her maternal half sisters, and her paternal half sisters were derived in general terms using Wright's methods of path coefficients *(11)*. Both the number of records per individual and the number of animals in a progeny group were retained in general form.

A general solution for the partial regression coefficients would have been so complex that the utility of the expressions for the partial regression coefficients would have been nil. Instead, the correlations for a given situation were evaluated and the partial regression coefficients were obtained from these. For evaluating the correlations, the basic statistics, which were computed ignoring year-to-year differences, were used since they more nearly represent what can be realized in a practical breeding program where an accurate adjustment for yearly effects is not often possible. The values used were: repeatability, 0.412; paternal half sister correlation, 0.120; maternal half sister correlation, 0.073; and heritability, 0.201. The genetic relationship between a sire and his mates was taken as zero and the genetic relationship between the mates of a sire was assumed to be 0.1 *(7)*.

When several records of the same cow are averaged, to get an expression for her phenotype, the assumption that each record was equally variable seems reasonable *(1)*. However, the assumption that each cow influences equally the average of a group of which she is a member is not tenable unless each cow has the same number of records. Where the numbers of records differ, the situation could be remedied by using only a single record for each cow (e.g., the first record); however, this would discard some of the available information. If cows with one, two, or more records are to be combined for a progeny sum or average, each cow's phenotype should be weighted in proportion to the reliability

with which her phenotype indicates her true producing ability. The variance of averages of n records is $\frac{1+(n-1)\,v}{n}$, times the variance of single records, where v is the repeatability of records of the same cow. Each cow's phenotype should be multiplied by the inverse of its variance $\left(\frac{n}{1+(n-1)\,v}\right)$ to be properly weighted. A convenient method of accomplishing this weighting is to express each cow's phenotype as her most probable producing ability. Lush *(4)* has shown this, expressed as a deviation from the herd average, to be $\frac{nv}{1+(n-1)\,v}$ (cow's average − herd average). Expressing each animal's phenotype in this way adjusts for the variation in number of records per individual, but the problem of weighting properly for the variation in numbers of individuals per sire or dam persists. This problem was met here by making solutions where information on one of the variables shifted, e.g., the number of daughters, but the information on the other variables remained the same.

The index derived was

$$I = X_1 + 0.4\,X_2 + b_3X_3 + b_4X_4 + b_5X_5$$

where X_1 and X_2 are the estimated real producing abilities of the cow and her dam respectively and X_3, X_4, and X_5 are the sums of the estimated real producing abilities of the cow's daughters, maternal sisters, and paternal sisters. These X's are all expressed as deviations from the herd average. Values for b_3, b_4, and b_5, the partial regression coefficients, are given in Table 5. The weights applied to X_1 and X_5 are given in a form which permits including the estimated real pro-

TABLE 5

Values of b_3, b_4, and b_5 for different numbers of daughters (n_3), maternal sisters (n_4) and paternal sisters (n_5) for computing index values

n_3	b_3	n_5[a]	b_5
1	0.385	1	0.053
2	0.365	2	0.079
3	0.336	3	0.070
4	0.314	4	0.063
5	0.288	5	0.057
		6	0.052
n_4	b_4	7	0.047
		8	0.044
1	0.099	9	0.041
2	0.093	10	0.038
3	0.087	11	0.036
4	0.081	12	0.034
5	0.077	13	0.032
		14	0.030
		15	0.029
		....	.....
		....	.....
		....	.....
		20	0.023

[a] The individual for whom the index value *(I)* is being computed is included in n_5; thus, when n_5 is equal to one, X_1 and X_5 are the same.

ducing ability of the cow being indexed in the sum for the paternal sisters. This eliminates the necessity of computing the sum for paternal sisters anew for each cow. Considerable complexity in the index is avoided by including each full sister simply as a maternal and again as a paternal sister. The values for X_3, X_4, and X_5 are expressed as sums, since the rapid increase in the standard deviation of the sum permits the partial regression coefficients to remain more nearly constant over the range in number of individuals (n_3, n_4, n_5) ordinarily encountered.

The genetic improvement in fat production anticipated from a given intensity of selection depends on the magnitude of the correlation between the breeding value for fat production *(G)* and the index *(I)* or basis of selection. When the index values approximate a normal distribution and truncation selection is practiced,

$$(G - \bar{G}) = r_{IG} \cdot \frac{z}{b} \cdot \sigma G$$

where z is the height of the ordinate of the normal curve at the point of truncation and b represents the fraction of the population saved for breeding. When selection is based solely on the information from the cow's own phenotype (mass selection), I becomes merely X_1.

The ratios of expected improvement from using the index in several situations, as compared with basing selections wholly on own performance, are shown in Table 6. Increasing the number of daughters (X_3) greatly increases the efficiency of the index. Practically, this is of little concern, since few cows in the herd have daughters in milk when selections concerning those cows must be made and these few generally have only one or two daughters. Increasing the number

TABLE 6

Ratios of expected progress from selections based on the index as contrasted to selections based solely on the cow's records for specific situations

No. records		No. individuals			rIG
X_1	X_2	X_3	X_4	X_5	rX_1G
3	3	1	1	5	1.092
3	3	2	1	5	1.116
3	3	3	1	5	1.162
3	3	4	1	5	1.188
3	3	5	1	5	1.208
3	3	1	1	5	1.092
3	3	1	2	5	1.094
3	3	1	3	5	1.096
3	3	1	4	5	1.098
3	3	1	5	5	1.099
3	3	1	1	1	1.088
3	3	1	1	5	1.092
3	3	1	1	10	1.094
3	3	1	1	15	1.095
3	3	1	1	20	1.096
1	3	0	2	5	1.184
1	3	0	2	10	1.203

of maternal half sisters (X_4) does little to increase the efficiency of the index. When the dam's own phenotype is well known, the cow's maternal half sisters, representing a progeny test of that dam, furnish little added information on the dam's breeding value. They would, of course, add more in case the dam herself had not been tested. The number of paternal half sisters (X_5) can be large in many instances and their influence can measurably increase the efficiency of the index, especially if the cow herself has only one record.

When a cow has as many as three records, as shown in the first part of Table 6, the new information provided by the relatives is limited. Correspondingly, the increase in efficiency of selection expected from using the index is small. In the last two lines of Table 6 are depicted situations where only one record is available on the cow and, hence, selecting on her record alone would make only about 0.78 as much progress as in the lines above. The relatives then provide relatively more new evidence about the cow's breeding value than if she already had three records of her own. Considering the extreme situations with conservatism, anticipated progress from using the index is 1.10 to 1.15 times that expected from basing selections solely on the cow's own performance.

The question arises as to how closely the average results from the study of a large volume of data apply in specific situations. For the present case, one might wonder if the estimates of the basic parameters actually are applicable to a particular herd. There seems to be no clear-cut method for determining whether these parameters actually do vary much from one herd to another. Furthermore, when the herds in this study were stratified into ten groups according to their average production, the daughter-dam regressions for the ten groups were not significantly different and they showed no trend. Where the herds are small, sampling errors alone may permit impossibly large or small results. Accuracy of prediction in a single herd for a short period may be low. However, the pluses in some herds and the minuses in other herds should cancel each other, so that the prediction of the average outcome in a whole breed or other population of many herds should reach a high level of accuracy. Even in a particular herd, the predicted and actual results should approach one another if the period is extended over several generations, provided the breeding system does not change extremely and suddenly.

The index developed in the present study considers only one item of economic importance, but presumably the most important one unless milk should be emphasized a bit more. The appearance of the cow also has economic value, but assigning an appropriate figure for the relative economic importance of type is an elusive problem. Disease resistance and tolerance to high environmental temperatures may merit inclusion in an index in some instances. Incorporating many traits and information on several relatives increases the complexity of the index. With dairy cattle, where the individual unit represents so much value and the generation interval is so long, more actual time spent in calculating indexes seems warranted than for most species of farm livestock.

Use of an index will do much toward making selection objective. Such a tool for selection is needed especially in dairy cattle where it is so easy for sentiment

to replace attention to records and sound judgment. Individual judgment cannot be replaced entirely by an index deduced from records which, at best, are incomplete measures of practical merit, but subjective judgment can well be relegated to a lesser role than it plays now. The index presented is only a beginning, but an index which would cover all situations and all pertinent traits would surely be unworkable because of its complexity.

SUMMARY

An index for intraherd selection for fat production was derived, utilizing the fat yields of the cow and those of her dam, daughters, maternal half sisters, and paternal half sisters. Statistics to construct the index were computed from 23,330 lactation fat yields of 12,405 Jersey cows on HIR test. The intraherd statistics needed to construct the index were: repeatability, 0.412; correlation between maternal half sisters, 0.073; correlation between paternal half sisters, 0.120; and heritability, 0.201. Herd differences accounted for 39% of the total variance. Within herds the year-to-year variation in things which affected all cows alike accounted for 8% of the variance.

Progress to be expected by using the index for selections would generally be about 1.10 to 1.15 times faster than by making selections on the cow's own performance alone. The exact ratio for a specific situation would depend on the number of records on the individual, on the number and kinds of relatives, and on the amount of information available about each.

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ANTIOXIDATIVE PROPERTIES OF CACAO AND THEIR EFFECT ON BUTTEROIL[1]

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Preliminary work *(2)* in 1938 revealed that cacao contained substances antioxidative for milk fat. Meager experimental data indicating the effectiveness of the cacao antioxidant in lard, soya bean oil, cod liver oil, and coffee beans are given in a patent *(3)*. Since the literature contained insufficient information to draw conclusions regarding the effectiveness of cacao antioxidants in dairy products, this investigation was undertaken. Possible antioxidants from cacao shell are emphasized in the present report.

PROCEDURE

The effectiveness of the cacao antioxidants on butteroil was tested by noting the formation of peroxides, bleaching of the carotenoid pigment, and taste and odor of the butteroil. Accelerated tests were used in place of ordinary storage conditions for dairy products. Although there is some risk of error in applying results obtained by accelerated tests, experience has shown that data thus obtained usually furnish a reliable guide to behavior during storage.

Preparation of butteroil. The butteroil for this study was prepared from sweet cream (approximately 40% fat) obtained from the University creamery. Cream was churned in a Dazey glass churn, and the resulting butter was melted and washed with water at 158° F. The oil and water were later separated by means of a separatory funnel. This process was repeated until a relatively clear butteroil was obtained. The final separation of oil and water was made by centrifuging in separatory funnels. The entire procedure was carried out away from direct sunlight. The butteroil contained 94.4% solids and was stored in amber-colored bottles in the refrigerator (42° F.).

Source of cacao. The cacao shell and cocoa powder were obtained from the Walter Baker Company, Dorchester, Mass. The cacao shell was ground to pass through a 20-mesh wire screen.

Swift fat stability test. The modifications of the Swift fat stability test as recommended by Riemenschneider *et al. (4)* were used with the exception that a 1-g. sample of butteroil was dissolved in 50 ml. of a mixture of glacial acetic

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acid and chloroform (3:2 by volume). All the peroxide numbers in this report represent the number of millimoles of peroxide present per 1,000 g. of fat.

Modified Schaal test. Twenty-gram samples of butteroil were incubated in unstoppered test tubes in the dark in an electrically heated hot air oven at 212° F. Before placing the test tubes in the oven and once each day thereafter, each tube was stoppered and then agitated by inverting it twenty-five times. The color of the butteroil was determined at various times.

Determination of color of butteroil. The yellow color of the butteroil or the degree of bleaching was determined by making comparisons with the aid of a Lovibond tintometer against 19 potassium dichromate dilutions, ranging from 0.10 to 0.001%. During the summer months, fresh butteroil has a color closely approximating that of 0.10% potassium dichromate; a concentration of 0.01% may be considered for all practical purposes as being completely bleached.

Taste and odor tests. Taste and odor tests were made by laboratory assistants and the writer. Odor was determined by smelling the tubes containing the oil, and taste was determined by transferring a small amount of oil by means of glass stirring rods from the tubes to the mouth. Since rancid taste and odor are only relative terms, their absence is indicated in the table by a negative sign and their presence by positive signs in three degrees of intensity.

RESULTS

Effect of varying amounts of copper on oxidative spoilage of butteroil. When the butteroil prepared for this study was aerated at 208.4° F. by the Swift test, the length of time necessary for the determination of its stability was approximately 50 hours. For obvious reasons, it is desirable to reduce the time for making this determination. It is well known that a metallic catalyst, such as copper, will greatly accelerate oxidative spoilage. Five samples of butteroil containing 0, 1, 2, 3, and 4 p.p.m. of added copper ($CuSO_4$), were aerated at 208.4° F., and peroxides and color were determined at various times. The time required to bleach the test butteroil samples to approximately 0.005% potassium dichromate color standard and the corresponding peroxide number at the time of bleaching are listed in Table 1.

Thus, the addition of 1, 2, 3, or 4 p.p.m. of copper to the butteroil greatly increased its susceptibility to oxidation. Since 4 p.p.m. of copper does not exceed

TABLE 1

Effect of varying amounts of copper on oxidative spoilage of butteroil

Amount of copper added	Time to bleach to approx. 0.005% potassium dichromate color standard	Peroxide No. when bleached to 0.005% potassium dichromate color standard
(p.p.m.)	*(hours)*	
0	51½	10.10
1	12½	9.39
2	9½	9.50
3	6	9.34
4	6	9.41

the maximum amount found in some dairy products, this amount of copper was added to butteroil tested by the aeration method in most instances in this study.

Effect of cacao shell on oxidative spoilage of butteroil with copper as pro-oxidant. Finely pulverized cacao shell depleted of fat by petroleum ether extraction was added to butteroil in the following concentrations: 0, 0.025, 0.05, and 0.10%. Four p.p.m. of copper (as $CuSO_4$) was added to all samples, which were then aerated at 208.4° F., and peroxide numbers, color, taste, and odor were determined at various times. Results obtained with 0.1% cacao shell are given in Table 2, and it is evident that cacao shell possesses considerable antioxidative properties toward butteroil. The antioxygenic effect of 0.025% cacao shell was found to be nearly equal to 0.05%, but less effective than 0.1% cacao shell.

TABLE 2

Antioxygenic influence of cacao shell in butteroil plus 4 p.p.m. of copper

Hours of aeration at 208.4° F.	Peroxide number	Color — % potassium dichromate	Degree of oxidized flavor
	Control		
0	0.00	0.10	−
4	1.08	0.10	−
5	1.11	0.10	+
8	3.05	0.07	++
8½	3.91	0.03	+++
9½	8.05	0.005	+++
	0.10% cacao shell added		
0	0.00	0.10	−
21	1.34	0.10	−
27	1.66	0.10	+
40	3.70	0.06	+
41	6.27	0.02	++
42	9.80	0.005	+++

Effect of cacao shell on oxidative spoilage of butteroil in absence of added copper. It is conceivable that a substance such as cacao shell may decrease the pro-oxidant effect of the accelerator (Cu) and yet have no direct effect on the oxidation of the natural fat. Consequently, the antioxidative properties of such substances could readily be misjudged.

Since copper was used as an accelerator in this study, a test was made to determine the antioxidative effect of 0.05% cacao shell on butteroil in the absence of added copper. The results are given in Table 3, and it is readily apparent that the cacao shell has definite antioxidative properties in the absence of added copper. However, its effectiveness is more pronounced when copper is added.

Effect of cacao shell, cocoa powder and extracts of these products on bleaching of butteroil subjected to a modified Schaal oven test. The purpose of investigating the antioxidative properties of various extracts of cacao shell and cocoa powder was two-fold: *(a)* to increase the potency of the cacao antioxidant by concentration and *(b)* to obtain the antioxidant in a form that could be more completely incorporated in the butteroil.

TABLE 3

Antioxygenic influence of cacao shell in butteroil without added copper

Hours of aeration at 208.4° F.	Peroxide number		Color—% potasium dichromate	
	Control	0.05% cacao shell	Control	0.05% cacao shell
0	0.00	0.00	0.10	0.10
42	2.53	2.39	0.08	0.08
50	6.21	2.57	0.02	0.08
53	11.48	2.96	0.001	0.07

In this experiment, cacao shell and cocoa powder and various extracts of these products were added to butteroil, and the stability of the butteroil was determined by the modified Schaal oven test, as described under *Procedure*. Rate of bleaching of color was the criterion for determining the antioxygenic properties of the various substances. The concentrations of the various substances and their effect on loss of color of butteroil are given in Table 4. It will be noted that the aqueous extracts of cacao shell were more potent than the defatted cacao shell. The temperature at which the aqueous extracts were made had no significant effect on the potency of the extract. The alcohol extract was just as effective as the aqueous extracts. The pro-oxidant effect of the ethyl ether extract may be due to the formation of peroxide during extraction, because it is difficult to maintain ether peroxide-free while making the extraction. Acetic acid extract of cacao shell had no antioxidative properties, but the sodium hy-

TABLE 4

Effect of extracts of cacao shell and cocoa powder on bleaching of butteroil subjected to a modified Schaal oven test

Substance tested	Concentration used	Time for bleaching[a] to a standard of 0.01% potassium dichromate
	(%)	*(days)*
Control		4
Cold water (42° F.) extract of cacao shell dried to powder	0.05	6
Hot water (145° F.) extract of cacao shell concentrated to 8.18% T.S.	0.05 on dry basis	6
Hot water (212° F.) extract of cacao shell concentrated to 11.20% T.S.	0.05 on dry basis	6
Hot water (212° F.) extract of cacao shell dried to powder	0.05	6
Hot alcohol (174° F.) extract of cacao shell concentrated to paste	0.05	6
Hot ethyl ether (95° F.) extract of cacao shell	0.05	3
Acetic acid extract of cacao shell	0.05	4
Sodium hydroxide extract of residue after acetic acid extraction	0.05	5
De-fatted cacao shell	0.05	5
Cocoa powder	0.05	6
Hot water (212° F.) extract of cocoa powder dried to powder	0.05	6

[a] Color of fresh butteroil is equal to approximately 0.1% solution of potassium dichromate and a 0.01% solution equals for most practical purposes completely bleached butteroil.

droxide extract of the residue after acetic acid extraction equaled the cacao shell in antioxidative properties. Cocoa powder was more potent as an antioxidant than cacao shell, and their aqueous extracts were of equal potency.

Effect of various substances on butteroil subjected to the Swift fat stability test. Defatted cacao shells were extracted in the following order in an attempt to isolate the cacao-tannin: chloroform, 5 days; ethyl ether, 6 days; and ethyl acetate, 5 days. According to Adam *(1)*, chloroform removes the theobromine and caffeine; ethyl ether removes the cacao-catechin; and ethyl acetate removes the cacao-tannin and probably phlobaphenes. All of the extracts were concentrated under partial vacuum and each was added to butteroil at a concentration of 0.1%. Also, the residue from the three extractions was added to butteroil at a concentration of 0.05%. Four p.p.m. of copper was added to all samples, including the control. All samples were aerated at 208.4° F., and peroxides, color, taste, and odor were determined at various times. The aeration time, peroxide number, and degree of color when a slight oxidized flavor was first noted and when a very pronounced oxidized flavor had developed are given in Table 5. It will be noted that the chloroform extract and the ether extract had a pro-oxidant effect. The ethyl acetate extract and the remaining residue after the three extractions possessed antioxygenic properties, but to a lesser degree than that of either dried water extract of cacao shell or cocoa powder. This indicates that not all the antioxidative material was removed from the defatted cacao shell by successive extractions with chloroform, ethyl ether, and ethyl acetate.

TABLE 5

Antioxygenic influence of various substances on butteroil subjected to the Swift fat stability test

Substance added		Slight oxidized flavor noted			Very pronounced oxidized flavor noted		
		Time aerated — hours	Peroxide number	Color — % potassium dichromate	Time aerated — hours	Peroxide number	Color — % potassium dichromate
Control		5	2.14	0.09	8	10.42	0.005
Caffeic acid	0.05%	>68[a]	1.90	0.10	>68[b]	1.90	0.10
Tannic acid	0.05%	69	1.56	0.08	>69[b]	1.56	0.08
NDGA	0.05%	32	2.27	0.10	>51[b]	3.33	0.10
Viobin	0.05%	19	2.10	0.10	22	12.05	0.005
Dried water extract of cacao shell	0.05%	29	2.25	0.09	39	10.33	0.005
Dried water extract of cocoa powder	0.05%	26½	2.20	0.08	36½	16.31	0.005
Chloroform extract of cacao shell	0.10%	<6[c]	7.62	0.007	6	7.62	0.007
Ethyl ether extract of cacao shell	0.10%	<6[c]	7.90	0.005	6	7.90	0.005
Ethyl acetate extract of cacao shell	0.10%	14	1.26	0.06	15½	11.52	0.005
Residue[d]	0.05%	19½	3.57	0.09	21½	11.03	0.009

[a] Maximum aeration time. Oxidized flavor absent.
[b] Maximum aeration time. A very pronounced oxidized flavor had not developed.
[c] A very pronounced oxidized flavor had developed.
[d] Residue after extracting with solvents in the following order: (1) petroleum ether, (2) chloroform, (3) ethyl ether, and (4) ethyl acetate.

In another experiment, the antioxygenic properties of cacao shell were compared with various antioxidants. A water extract of cacao shell dried to a powder was selected for making the comparison with the following antioxidants: caffeic acid, tannic acid, nordihydroguaiaretic acid (NDGA) and Viobin. All substances were added to butteroil in concentrations of 0.05%. Four p.p.m. of copper was added to all samples, including the control. The samples were aerated at 208.4° F., and peroxide, color, taste, and odor were determined at various times. The aeration time, peroxide number, and degree of color when a slight oxidized flavor was first noted and when a very pronounced oxidized flavor had developed are given in Table 5.

In general, the dried water extract of cacao shell was more effective in retarding oxidative spoilage than Viobin but less effective than caffeic acid, tannic acid, and NDGA. However, as judged by the first appearance of a slight oxidized flavor, the dried water extract of cacao shell nearly equaled NDGA in antioxygenic properties. The water extract of cacao shell had an advantage in that it did not impart an objectionable flavor to butteroil as did caffeic acid, tannic acid, and NDGA, in the concentrations used.

SUMMARY

The antioxidant properties of cacao shell, cocoa powder, and certain extracts of these products were determined in butteroil by the use of two accelerated tests, namely, the modified Schaal test and the Swift fat stability test. Determinations were made for peroxide values, color, taste, and odor.

This study substantiated the writer's previous (1938) observations that cacao shell and cocoa powder contain a natural antioxidant that is effective in retarding oxidative deterioration of milk fat. The cacao antioxidant was more effective when copper was added. The cacao antioxidant also was an inhibitor of the prooxidant effect of copper.

The antioxidants in cacao shell were partly extracted with water or alcohol, and the concentrated water and alcohol extracts were more potent than cacao shell or cocoa powder. Drying the water and alcohol extracts to a powder did not significantly decrease their protective properties. In general, the dried water extract of cacao shell was more effective in retarding oxidative spoilage than Viobin but was less effective than caffeic acid or tannic acid. The dried water extract of cacao shell nearly equaled NDGA when the antioxygenic effect was evaluated by comparing the aeration time to produce the first slight oxidized flavor. The antioxidants in cacao shell and cocoa powder were not extracted with fat solvents.

A cacao tannin-like material was isolated from cacao shell and was found to be less potent as an antioxidant than pure tannic acid.

The cacao inhibitor at a concentration of 0.05% did not impart a foreign flavor to the butteroil.

Whether the antioxidative materials derived from cacao are nontoxic has not been determined.

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STANDARDIZED PROCEDURES FOR THE BABCOCK TEST FOR MILK

Report of the Committee of the Manufacturing Section of A.D.S.A.

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A study of *Official and Tentative Methods of Analysis* of the Association of Official Agricultural Chemists reveals no significant refinements in procedures for the Babcock test during the past 35 years. In fact, procedures for all phases of sampling and testing milk have never been stated in detail by the Association. This is not intended as a criticism of the Association, as the dairy industry has not, until recent years, demanded more refined and detailed techniques.

The Babcock test is unique among chemical tests in that it is subjected to variable techniques in different states. These include the most important procedures in the test, such as *(a)* methods of sampling milk from weighing tanks; *(b)* temperatures for preparing, handling, and storing fresh and preserved composite samples; *(c)* sampling and pipetting temperatures for fresh and preserved composite samples; *(d)* temperatures in centrifuges and in water baths; and *(e)* reading the fat columns. It is unlikely that any other important chemical test is subjected to so many variations imposed by statutes and traditional practices. These variables provide grounds for disputes that may involve court litigation and thereby disrupt good public relations among producer, dealer, and consumer groups.

The desirability of standardizing the test is obvious in the light of these facts:

1. Dealers and handlers use the test as a basis for paying producers or marketing organizations for their milk.

2. They also use the test as a basis for selling milk and cream and other products to other dealers.

3. Operators of milk plants need to know the fat content of their products in order to check the efficiency of their operations.

4. Variations in methods and techniques make it difficult, if not impossible, for any agency, public or private, to verify the accuracy of tests of milk fat and other milk products on a legal basis.

5. Milk and cream are shipped in interstate commerce.

At the annual meeting of the American Dairy Science Association in 1946, a subcommittee was appointed to evaluate the status of the Babcock test and to determine whether it had kept abreast of other technological advances in the

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dairy industry. At the suggestion of industry groups and regulatory agencies, similar action was taken by the Association in 1938, but the study was suspended during the war. The subcommittee appointed in 1946 set out to obtain the facts from the proper agencies in all states. The department of agriculture, board of health, or other responsible regulatory agency in each state was asked for a copy of its laws pertaining to the testing of milk. In some states the agency sent agricultural college publications, which served as state regulations. The committee tabulated the techniques used in sampling and testing milk in each state and returned the data to the state for verification. An abbreviated summary of this study *(1)*, reported in 1947, showed much variation in sampling and testing milk. Some states approved techniques which were prohibited by others. It was the opinion of the committee that some of them were wrong and others were unnecessary. Recently *(2)* the study of 1947 was brought up to date with a discussion of techniques of the test as conducted in the different states.

As a result of its study, the subcommittee *(1)* recommended that standardized procedures for all phases of the Babcock test be formulated and submitted to the A.D.S.A. for consideration. In 1950, Dr. G. H. Wilster of Oregon State College was appointed chairman of a committee to formulate detailed procedures for the test, and he continued as chairman until 1952, when he resigned because of illness. During his chairmanship much work was done which provided a basis for the final report. In 1952, Mr. Burdet Heinemann assumed chairmanship of this committee and is responsible for the final draft of procedures, which are based on the best experimental evidence and experience at the present time. They were approved by the executive committee of the A.D.S.A. at the University of Wisconsin in June, 1953. This is the first time a unified set of procedures for all phases of the Babcock test has been formulated on a national basis.

The sampling procedures specified in this report can be applied also to total solids and other chemical determinations for milk. The procedures follow.

I. *Weighing milk*

A. The weigh tank shall be of such size and shape as to completely mix all the milk. If it is not of this type, it shall be equipped with a mechanical agitator. It shall be maintained in a satisfactory mechanical and sanitary condition, free from dents or bulges which may prevent adequate draining.

B. The scales shall be checked daily with test weights. Twice each month the scales shall be checked for accuracy at the maximum capacity of the weigh tank.

II. *Sampling milk from the weigh tank*

A. Mixing or stirring before sampling

1. The milk shall be thoroughly mixed to insure the withdrawal of a representative sample for testing.

2. Verifying mixing efficiency

a. The method of securing a representative sample shall be verified at least monthly on several shipments of milk.

b. The mixing efficiency of the weigh tank shall be determined by taking samples, simultaneously, from each of the four corners and the center and testing them in duplicate. If the averaged tests of these samples differ consistently by more than 0.1% fat, then the mixing efficiency shall be considered unsatisfactory.

c. If the mixing efficiency is not satisfactory as indicated by results obtained in section *b*, then each lot of milk shall be stirred vigorously with a hand stirrer for one minute prior to sampling.

d. The accuracy of sampling milk which is not to be dumped in a weigh tank shall also be verified monthly by taking 5 samples at different time intervals of mixing or from different sampling locations of the same lot of milk.[8]

3. Abnormal milk

a. All milk dumped into the weigh tank shall be sampled unless it is severely frozen. The producer should be notified promptly if the milk is frozen, churned, or otherwise unsuitable for thorough mixing, and corrective measures shall be taken.

III. *Care of composite samples*

A. Preservative

1. A preservative containing color shall be added to milk samples if they are held more than 24 hours. Formalin (36% solution of formaldehyde) may be added at a rate not to exceed 0.1 ml. for each 30 ml., or 2 drops per fluid ounce of milk, or tablets containing a preservative of proven efficiency may be used provided that the weight of the tablet or tablets does not exceed 0.5 g. per 8-oz. bottle.

B. Period of composite sampling

1. Composite samples shall be collected over a period not to exceed 16 days.

2. Composite samples shall be tested within 24 hours after the last addition of milk except when other arrangements have been made with the proper regulatory agency.

C. Quantity of milk to be taken

1. A minimum of 10 ml. shall be taken from each producer's delivery of milk. The quantity taken shall be the same for each day during the composite period.

[8] This refers to tank trucks, storage tanks, pasteurizers, etc.

2. The total composite sample at the time of testing shall be not less than 100 ml. If, for example, a producer delivers milk only 2 days during a composite period, then a minimum sample of 50 ml. must be taken on each of the 2 days.

D. Size of sample bottle

1. Composite sample bottles shall have a capacity of not less than 8 oz. and shall be free from cracks and tightly fitted with sanitary rubber stoppers.
2. Every bottle shall have a legible and permanently attached mark of identification.
3. Every sample bottle shall be clean and dry before the addition of a preservative and shall be maintained in such a manner as to prevent the accumulation of moisture which may dilute the milk.
4. The milk plant shall have at least two complete sets of sample bottles.

E. Method of sampling

1. When a dipper, tube, or similar manual device is used, it must be rinsed once with the milk to be sampled.
2. When an automatic device is used, evidence must be available to verify the adequacy of rinsing the device with the milk to be sampled.
3. If the weigh tank does not hold all the milk from one producer, it may be divided into two approximately equal portions and the same sized sample taken from each portion. This requires two composite sample bottles. This must be done during the entire composite period even though the volume of milk delivered daily might decrease to one full weigh tank. The two composite samples may be mixed prior to testing, or each bottle may be tested and the test averaged for payment.
4. The composite samples should be gently rotated after the addition of each daily sample. Rough handling of trays or bottles should be avoided. Breakage or spillage should be avoided, but if either occurs, a permanent record must be made showing the date of accident and identity of the sample.

F. Storage conditions

1. The composite samples shall be protected from light and stored in a clean, sanitary cabinet maintained at 35 to 50° F.
2. The samples shall be kept at room temperature for not longer than 1 hour each day during the composite period.

G. Period held after testing

1. All milk samples from completed and recorded tests shall be held at 35 to 50° F. for at least 5 days after testing.

IV. *Preparing both fresh and composite samples for testing*

A. Tempering

1. Place the sample bottles in a water bath with the surface of the water slightly above level of the milk in the bottles.
2. The temperature of the bath should not exceed 110° F. at the time the cold samples are placed in it. Shortly thereafter it may be adjusted, as the temperature of the milk rises, so that the final temperature of the bath and the samples is between 90 and 100° F. Two baths may be used, one for heating and one thermostatically controlled for holding at 90 to 100° F. Do not shake or mix the milk in the sample bottle until it has reached 90° F.
3. Any sample bottle which may become diluted with water must be discarded and a permanent record made of the date of accident and identity of the sample.
4. Any cream adhering to the sides of the bottle and the stopper must be reincorporated with the contents by gently rotating and inverting the bottle. A special tool with a rubber disk may be used to dislodge the adhering cream if necessary.[9]

B. Mixing before pipetting

1. The composite sample, after reaching 90 to 100° F., is poured into a mixing container and back into the original sample bottle at least two times. The mixing container shall be drained at least 15 seconds before using it again.
2. If the composite bottle is not over two-thirds full, the sample may be mixed by shaking it horizontally back and forth six times through a distance of about 6 in. within about 3 seconds, being careful to avoid churning.
3. The sample shall be pipetted immediately after mixing.

C. Temperature of milk for pipetting

1. The temperature of the milk at the time of pipetting shall be between 90 and 100° F.

D. Pipetting

1. The tip of the pipette should be placed into about one-half the depth of the milk in the sample bottle. It is then filled and the topmost surface of the milk is adjusted to the graduated mark on the suction tube. The milk is then discharged by inserting the entire delivery tube into the neck of the test bottle before releasing the contents. The lip of the test bottle should be vented, preferably with a grooved rubber gasket at the base of the bulb of the pipette.

[9] Available from Collins Dairy and Food Laboratories, Dryden, N. Y.

2. When the pipette has drained for about 10 to 15 seconds after free flow has stopped, blow out the last drops or remove it from the test bottle with a quick upward movement to force the last drops from the tip. Two pipettes, used alternately, will be advantageous.

V. *Testing milk for butterfat*

A. Sulfuric acid

1. The sulfuric acid shall have a specific gravity of 1.82 to 1.83 at 68° F.
2. The temperature of the acid shall be between 65 and 75° F.
3. The amount of acid used shall be such that the fat column of the finished test will be clear and free from foreign material. (This amount shall be not less than 14 ml.)

B. Adding acid to the milk

1. The temperature of the milk at the time of adding the acid shall not exceed 80° F.
2. The test bottle shall be held at an angle and the acid added slowly all at one time. The blending of milk and acid should be avoided until the actual mixing begins.
3. The mixing of milk and acid should be started gently by rotating the test bottle until the curd disappears. Continue shaking for an additional period of at least 30 seconds. The time of shaking is very important in obtaining accurate tests. If formaldehyde is used as a preservative, mixing for 3 minutes is recommended.[10]
4. The bottles should be placed in a heated centrifuge immediately after mixing milk and acid.

C. Centrifuge

1. The centrifuge shall be thermostatically controlled at 140 to 150° F. while in operation. An accurate thermometer shall be permanently attached to the centrifuge in such a manner that it will indicate the temperature in the area where the test bottles are whirled.
2. The centrifuge shall be mechanically driven, free from vibration, and at the following speeds:

Diameter of wheel in inches	*Minimum r.p.m.*
16	848
18	800
20	759
22	724
24	693

[10] A mechanical shaking device may be used.

Diameter of wheel means the distance between the inside bottom of the opposite cups, measured through the center of rotation when the cups are extended horizontally. This diameter shall not be less than 16 in.

3. The speed of the centrifuge shall be checked at least monthly and shall be not less than the figures in the preceding table and not exceed them by more than 40 r.p.m. The speed shall be determined with the door of the tester closed. An accurate tachometer permanently attached to the centrifuge is desirable.

D. Centrifuging procedure

1. The centrifuge shall be operated 5 minutes for the first period, 2 minutes for the second, and 1 minute for the third. Timing shall begin after the operating speed is reached. A satisfactory timer shall be used.
2. As an alternative method, the centrifuge may be operated 5 minutes for the first period and 3 minutes for the second. This requires only one addition of water to bring the fat into the neck of the bottle.
3. Soft water, free from oil, and at least 140° F., shall be used for filling the bulb and adjusting the level of the fat column in the neck of the test bottle.
4. The test bottles shall be transferred to the water bath immediately after the final centrifuging period.
5. At the time of reading, the entire fat column must be within the graduated neck of the test bottle.

E. Tempering baths

1. The water in the bath shall be between 130 and 140° F., preferably 135° F.
2. The water shall be kept slightly above the top of the fat columns of all test bottles.
3. The test bottles shall remain in the water bath for a period of not less than 3 minutes.
4. Tests shall be read immediately after removal from the water bath.

F. Reading tests

1. Sharp needle-pointed dividers or calipers shall be used for measuring the fat column. The bottle shall be held vertically and at eye level in front of a source of indirect light at the time of measuring. The use of a 5-inch magnifying lens in reading the fat column is recommended.[11, 12]

[11] A useful mechanical caliper for reading Babcock tests, equipped with a magnifying lens and a source of white light, is available from the Windsor Company, Glen Ellyn, Ill.

[12] For a source of light only, the fluorescent titration illuminator is useful; it is manufactured by the Fisher Scientific Co., St. Louis, Mo.

2. The fat column shall be measured in its entirety from the bottom of the lower meniscus to the top of the upper meniscus. (The meniscus is the saucer-shaped surface at the ends of the fat column. The bottom of the meniscus is the bottom of the saucer, and the top of the meniscus is the outer rim of the saucer.)
3. The fat column shall be measured on the ungraduated side of the neck and the test read from the graduated scale.
4. The fat test shall be read to the nearest 0.1%.
5. Tests in which the fat column is foamy, burnt, or curdy shall not be read.

VI. *Glassware*

A. Calibrated glassware used in making the Babcock test shall conform to the minimum standards set forth in the latest edition of *Official Methods of Analysis* of the Association of Agricultural Chemists, or any supplement thereto.

NOTE

In the event that the American Dairy Science Association modification of the Babcock test *(3)* is adopted by the Association of Official Agricultural Chemists, the following changes in this report are necessary:

1. Pipette (Section IV-D-1). The A.D.S.A. modification requires a pipette calibrated to contain 18.05 ml. of water at 95 to 97° F. when the bottom of meniscus coincides with mark on suction tube.

2. Reading the fat column (Section V-F-2). The A.D.S.A. modification requires the addition of two drops of glymol before reading the test. The fat column is then measured from the bottom of the lower meniscus to the fat-glymol line.

REFERENCES

(1) Heinemann, B., Herreid, E. O., Josephson, D. V., England, C. W., and Swope, W. D. A Study of Procedures Used in Conducting the Babcock Test in the Various States. *J. Dairy Sci.*, 30: 963. 1947.

(2) Herreid, E. O., and Heinemann, B. Techniques Used in the Babcock Test for Milk in the United States. Ill. Agr. Expt. Sta., *Circ.* 709. 1953.

(3) Herreid, E. O., Burgwald, L. H., Herrington, B. L., and Jack, E. L. Standardizing the Babcock Test for Milk by Increasing the Volume of the Sample and Eliminating the Meniscus on the Fat Column. *J. Dairy Sci.*, 33: 685. 1950.

PROPOSED PUBLIC RELATIONS OBJECTIVES OF THE AMERICAN DAIRY SCIENCE ASSOCIATION

A COMMITTEE REPORT

A. *General Objective of the Association:* (Article II, Section 1 of the Constitution). "The object of the Association shall be to stimulate scientific research in all phases of interest to the dairy industry and to dairy science, to improve methods of dairy instruction and extension work, to cooperate in educational developments in advancing the general welfare of the dairy industry, and to make available through the *Journal of Dairy Science* the results of the latest scientific research pertaining to these fields." *Supplemental Note:* The object of the Association as presented in the Constitution indicates that the basic purpose of the Association is to provide an organization in which persons having common interest in the science of dairying may unite to promote the general welfare of the Dairy Industry through cooperative effort, and through which educators, research workers, industry and farm leaders in the dairy field may pool and disseminate scientific knowledge by means of organized meetings, official publications, and effective teaching practices.

B. *Specific Objectives for the External Public Relations of the Association:*

1. To develop and maintain organizational programs and policies of such high value as to command support of all phases of the Dairy Industry and to justify active membership in the Association by qualified dairy leaders in education, research, and industry.
2. To utilize a dynamic approach to acquaint the members of the Dairy Industry with the activities of the Association and thus to insure satisfactory membership in the Association and subscriptions to the *Journal of Dairy Science*.
3. To assume the responsibility and the initiative for sponsoring and/or supporting educational programs in the dairy field which are developed *(a)* for individual and group improvement through the free interchange of scientific information, and *(b)* for creating a more enlightened public in regard to the Dairy Industry.
4. To promote cooperation of the Association and of the various branches of the industry in specific endeavors for furthering the advancement of dairy science, the welfare of the industry, and the interests of the public.
5. To encourage and give recognition to research, to make available prompt means for reporting research findings, and to assist in interpreting the research results in terms of practical application.
6. To stimulate continuous improvement in teaching techniques, methods, and programs within and outside of the classroom and to encourage frequent review of these for the purpose of maintaining a high degree of teaching effectiveness in the face of rapidly changing conditions.

7. To improve communication between all branches of the dairy industry in order *(a)* to shorten the time between research results and application, and *(b)* to prevent internal misunderstanding resulting from incorrect or insufficient information.

8. To develop, cooperatively with industry groups, appropriate programs for insuring a continuing supply of college graduates, well trained in dairying and in science and endowed with leadership qualities.

9. To appraise frequently the *Journal of Dairy Science* in the light of the objectives of the Association to ascertain if it is contributing the benefits and rendering the services desired by the membership and to initiate innovations or alterations in the *Journal* which are indicated clearly by objective membership surveys.

C. *Specific Objectives for the Internal Public Relations of the Association:*

1. To create and maintain an atmosphere of warmth and friendliness in the Association; to make each person feel that he/she is an important part of the Association and therefore should exercise full membership privileges in participating freely in all Association activities and in the making of its policies; to give continued attention to the varied special interests of the individual members so as to avoid over-emphasis in one area at the expense of another.

2. To develop and utilize effective procedures for indoctrinating new members in the Association and for keeping membership informed at all times of action taken by the Executive Board relating to the policies and practices of the Association.

3. To design the business session of the annual meeting with the view of making it highly informative and interesting to the members; to prepare and distribute an agenda which will provoke discussion by individual members and arouse their interest; to reduce to a minimum stereotyped reports presented for traditional reasons rather than for real value; to encourage the presentation at the annual business meeting of sectional reports which represent the views and major recommendations of the sections and their officers for the current year and which would be of general interest to the membership.

4. To establish procedures for the handling of committee reports which are *(a)* comprehensive and long, and/or *(b)* on controversial subjects, so that members will have an opportunity to study the reports well in advance of the time for formal action.

5. To devise and conduct a progressive program to sample the opinion of the membership periodically on timely matters pertaining to Association affairs and to the *Journal of Dairy Science* and to use the results as a basis for planning for the future.

6. To provide means of maintaining close inter-relationship between the Sections of the Association and the Executive Board; to establish clear channels of communication between all groups so that full understanding will prevail of programs, policies, practices, and problems of mutual interest.
7. To develop for general use manuals which will include the procedures and practices of each Section, those which involve a relationship between the Sections and the Executive Board, and those which pertain to standing committees of the Association.
8. To establish clear-cut policies in regard to the treatment of Sectional and Association Committee reports and to define the respective responsibility and authority of a Section and/or the Executive Board relative to official approval, reproduction, and distribution of such reports.

F. J. Arnold
H. A. Bendixen
R. E. Frost
H. B. Henderson
G. E. Holm
I. A. Gould, *Chairman*

Committee on Public Relations

PEOPLE and EVENTS
in the Dairy Science World

Idaho Milk Processors Association Holds District Meetings

The annual district meetings of the Idaho Milk Processors Assoc. were held at Pocatello, Twin Falls, Caldwell, and Moscow, on April 5, 7, 9, and 15, respectively. The programs included papers and discussions on "The Keeping Quality of Dairy Products," "The Costs of Milk Production," "Bulk Handling of Milk on the Farm," "Merchandising Milk in Gallon Jugs," "Cleaning Pipe-Line Milkers," and "State Dairy Regulations."

Dairy products scoring clinics were conducted in the afternoons at Caldwell, Twin Falls, and Moscow by J. C. BOYD, of the Dept. of Dairy Husbandry, Univ. of Idaho.

Officers of the association who arranged for the meetings are: M. L. PARROTT (Dairymen's Coop. Creamery of the Boise Valley, Caldwell), president; TOM SPEEDY (Young's Dairy, Twin Falls); FERD JAUSSI (Bear Lake Coop. Creamery, Paris), and ARVID JOHNSON (Van's Creamery, Coeur d'Alene), directors. Boyd is secretary-treasurer of the association.

Judges Named for '54 International

Six well-known judges of dairy cattle comprise the slate of those who will officiate at the 2nd annual International Dairy Show, October 9 to 16, in the International Amphitheatre, Chicago. Ayrshires will be judged by R. C. FOLEY, Univ. of Massachusetts; Brown Swiss by HAROLD KAESER, Ohio State Univ.; Guernseys by W. K. HEPBURN, JR., of Forsgate Farms, Jamesburg, N. J.; Holsteins by G. W. TRIMBERGER, Cornell Univ.; and Milking Shorthorns by J. E. OAKLEY, Uniontown, Ala.

The National Jersey Show, to be held in Chicago this year, will have a two-man judging committee—DALE DEAN, of Ridgway, Mich., and HAROLD KAESER. The same judges will also pass on the junior classes of each breed. The junior classes are scheduled October 11, and the open class judging of the six breeds will follow through the week. First and second prize winners in the Junior Show will automatically be permitted to compete in the open show, according to W. E. OGILVIE, manager.

Officials of the show report that construction of a 180,000 sq. ft. addition to the International Amphitheatre will soon be under way. When completed next fall it will be the nation's largest exhibition building. The addition will extend 600 ft. south of the present Amphitheatre, fronting on Halsted Street.

Maine's Annual Farm and Home Week

The 47th annual Farm and Home Week Program was held April 5-8 on the Univ. of Maine Campus. This program honors outstanding Maine farmers and homemakers and brings to the residents of the state a varied program in the fields of agriculture and homemaking. Out-of-state speakers on the dairy program were: ROBERT SINCLAIR, Univ. of Vermont; W. P. DAVIS, New England Milk Producers' Assoc., Boston; C. F. HUFFMAN, Michigan State College, and M. P. JONES, USDA, Washington, D. C.

Southern Division, A.D.S.A., Meets in Dallas

The 27th annual meeting of the Southern Division, A.D.S.A., was held in Dallas, Tex., on February 1, 2, and 3, 1954. Thirty-one papers were presented. The officers for 1954-55 are: S. P. MARSHALL, president, Univ. of Florida; W. A. KING, vice-president, Clemson College; H. C. OLSON, secretary-treasurer, Oklahoma A & M; and directors—C. L. NORTON, Oklahoma A & M, and C. W. REAVES, Univ. of Florida. J. P. LAMASTER of Clemson College was presented the honor award for 1953-54 at the banquet.

Happenings in South Carolina

A milk plant fieldman's conference was held at Clemson College on March 17 and 18. The program included discussions on dairy farm planning, management, feeding, and better breeding methods. A feature of the program was instruction in milk and cream judging. Nineteen fieldmen, representing 14 plants in the state, attended the conference.

A Mellorine bill was considered by the South Carolina legislature which would legalize the use of certain vegetable fats in frozen desserts. The bill was not passed.

The South Carolina legislature has enacted a dairy "Audit and Import Law." A dairy commission established is charged with the enforcement of the law. The commission is made up of nine members, three milk producers, three milk distributors, two representatives of the consuming public, and the Commissioner of Agriculture, ex-officio. J. E. CUSHMAN has been employed as director.

O.S.U. Dairy Technology Career Day Attracts Many High School Students

One hundred six high school seniors attended the first Dairy Technology Career Day held at the Ohio State Univ., April 24. Dairy industry representatives visited high schools in their local areas and contacted students interested in visiting the campus and the Dept. of Dairy Technology. Thirty-five industry representatives furnished transportation for the students from their homes to the campus.

The program included a tour of the dairy technology laboratories and plant, the showing of the film "The Miracle of Milk," a discussion of the training and opportunities in the field of dairy technology, a film, "The University in Action," and a luncheon at Pommerene Hall. The formal program was concluded with a discussion by representatives of the industry of the need for trained men in dairy technology. The visitors then toured the campus or attended the Ohio State-Indiana baseball game.

News from the Deep South

An artificial insemination training course for technicians, sponsored by the Dairy Dept. of the Louisiana State Univ., was held recently. Twenty-three persons took the 1-week course. Louisiana now has 43 local breeding circuits employing 52 technicians. Last year 37,584 cows were inseminated, 40% to Jersey bulls, 25% to Holstein, 25% to Guernsey and 10% to beef bulls. This showed an increase for Guernsey and Holstein and decreases for Jersey and beef bulls from the previous year.

E. J. STONE was recently appointed assistant professor in dairying at L.S.U. He comes from the North Louisiana Hill Farm Experiment Station at Homer, where he was in charge of the dairy section. In 1947, after service with the Air Force, Mr. Stone was graduated from Rutgers Univ., receiving a B.S. degree in animal husbandry. In 1949 he received his M.S. degree from the same institution. In 1949-1951, he served as instructor and later as assistant professor of dairy husbandry at the North Dakota Agricultural College. Before coming to Louisiana he was managing a large dairy farm in New York State.

Louisiana will soon have a $500,000 milk processing plant capable of converting 200,000 lb. of raw milk a day into salable milk products. This plant will be the first to be built under provisions of Louisiana's Industrial Inducement Act of 1952 and is reported to be the first of its kind in the South. Funds will be raised through the sale of tax bonds by Washington parish, these bonds to be retired by revenues from the business. It will serve 1,400 farmers in the New Orleans milk shed. This plant will be able to handle the surplus production that the New Oreleans fluid milk market can not use, converting it into nonfat dry milk solids, whole milk powder, condensed milk, sweet cream, butter, or cheese.

To help Louisiana families financially and nutritionally, a series of milk utilization meetings were held during 5 days in March. MISS RITA DUBOIS, Regional Consumer Marketing Specialist, USDA, assisted by MISS MCQUEEN, MISS COOLEY, and MR. NEASHAM, all of the L.S.U. Extension Dept., met with milk processors, their drivers, and home demonstration agents in five parts of the State. Nearly all of the parishes were represented by the agents. Miss Dubois demonstrated various uses that could be made of milk. Discussions followed the sessions. At luncheon, the processors, who were the hosts, were told what they could do to increase milk utilization. And to the drivers was given a special talk on what they could do in their contacts with customers to promote sales.

BUCK GREEN assumed the duties of research associate in dairy husbandry, Southeast Louisiana Dairy and Livestock Experiment Station on March 3, 1954. He was formerly employed as research associate in the L.S.U. Dairy Dept. Mr. Green completed the requirements for B.S. and M.S. degrees in agriculture at L.S.U.

A.D.S.A. Public Relations Meeting

The committee appointed by President PRICE to consider the proper public relations objectives of the A.D.S.A. will give a brief report at the business meeting of the Association at the time of the annual meeting at Penn State. Members are urged by Chairman I. A. GOULD to be present for the discussion of the report. Three members of the Association representing different areas of interest have been invited to give their views on ways to make the Association more effective.

Wisconsin Completes New Dairy Buildings

A new dairy cattle instruction and research center has been completed at the Univ. of Wisconsin. Instruction in dairy husbandry can now be given under the same roof where the herds are kept. The barn itself is in two 40 by 140 ft. wings joining a brick and glass center section 76 by 60 ft. One wing is to be used for the instructional herd, and the other is for research work on dairy cattle. The two-story center section holds the modern four-stall milking parlor, offices, class room, and utility space.

New Faculty Member at Davis

L. M. SMITH has joined the faculty of the Dept. of Dairy Industry, Univ. of California, as assistant professor and assistant dairy technologist in the Experiment Station beginning May 1. Smith was formerly in the Dept. of Dairying, Univ. of Alberta, Edmonton, Canada.

Cornell Holds "Honors" Meeting

E. R. EASTMAN, editor of *American Agriculturist*, was honored May 8 along with three outstanding students in dairy courses at the fourth annual awards banquet of the Cornell Dairy Science Assoc. Eastman received an engraved bronze plaque for his "great and lasting contributions to the dairy industry in New York state." Students who received dairy science keys and $25 were RONALD MILLER of Sauquoit, HARVEY BREIER of Long Island City, and DAVID BANDLER of New York City. Speaker of the evening was BO ADLERBERT, president of Sunrise Dairies, Hillside, N. J. Miller, a freshman, and Breier, a sophomore, were recognized for their interest and enthusiasm in the Dairy Science Association activities. These two awards are open only to freshmen or sophomores in the 4-year course. Bandler, a junior, was honored for his scholastic work and leadership ability as shown by extra-curricular activities.

On behalf of the College of Agriculture, Dean W. I. MYERS accepted a new Cornell Dairy Science Assoc. scholarship of $300 to be given to any student in the university who is enrolled in the dairy industry course in his sophomore year who excels in leadership and scholarship and shows financial need. Purpose is to encourage and make it possible for a qualified student to enter the training program in dairy industry.

Oregon Events

ANTONE (TONY) LARSEN of Sunshine Dairy, Portland, again headed the Oregon June Dairy Month campaign. "Booster" tours through the state were made by Larsen and his group to meet with local service organizations and dairy leaders, to stimulate local interest, and to get better understanding of the dairy industry.

A record high of 37,133 cows were tested on DHIA in Oregon last year, according to H. P. EWALT, dairy extension specialist. The number of herds tested was up 3%, and 6½% more completed the testing year. The average production per cow was 8,294 lb. of milk and 380 lb. of milk fat.

The Wandamere Guernsey herd, owned by M. C. FLEMING, Troutdale, was purchased last month by A. R. and ALVIN C. BUSH of Wyno Farms, Muncy, Pa. This was one of the largest interstate herd sales on record, with more than 100 head of registered Guernseys involved.

Completed Theses

M.S. Degree:

C. C. HYDE—Effect of type of protein on the response of young dairy calves to aureomycin. Louisiana State Univ.

BUCK GREEN—Studies of the effects of summer climatic conditions on physiological reactions, semen production, and fertility of bulls used for artificial insemination in Louisiana. Louisiana State Univ.

E. B. MORGAN—The metabolism and fertility of bovine spermatozoa in egg yolk-citrate and milk diluents. Louisiana State Univ.

BOBBY L. KIRKPATRICK—The effect of different ratios of feeding grain on milk production of dairy cows. Louisiana State Univ.

BENJAMIN F. MAGILL—The relationship of preejaculation stimulation to some of the physiological and morphological characteristics of semen from identical twin bulls. Oregon State College.

JOHN V. BATEMAN—The fermentation losses, changes in bacterial flora, and acidity of grass silage preserved with dried molasses beet pulp and sodium metabisulfite. Oregon State College.

Ph.D. Degree:

JOHN H. BYERS—The influence of carotene levels in dairy cattle rations on reproductive behavior, feed utilization, and other physiological processes. Oregon State College.

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JOURNAL OF DAIRY SCIENCE

ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

ANIMAL DISEASE

579. A comparison of individual can and weigh-vat sampling for the Brucella ring test. G. T. WOODS, Univ. of Ill., Urbana. N. Am. Veterinarian, **35**, 2: 103. 1954.

This work was done to determine if weigh-vat contamination with infected milk is responsible for false positive reactions to the ring test. Duplicate can and weigh-vat samples are reported on 527 Illinois producers. Samples were taken from each can and pooled. After the milk was dumped into the weigh vat, a second sample was taken. Milk samples were tested by the ABR test. There was disagreement between the sampling procedures in 63 (12%) of the producers' samples. Eighteen of these samples showed negative ring tests on can collected samples, but positive on vat samples. In all 18 instances the preceding weigh-vat delivery was positive. This shows an advantage of collecting samples from cans rather than from the weigh-vat. R. P. Niedermeier

580. Treatment of chronic bloat with antibiotics. A preliminary report. W. H. CHIVERS, Iowa State Coll., Ames. N. Am. Veterinarian, **35**, 3: 186. 1954.

The author observed several clinical cases where animals were treated with aureomycin for acute infections, and chronic bloat that also happened to be present disappeared. A study then was set up specifically to test the treatment of chronic bloat in young cattle with antibiotics. Treatment consisted of intravenous administration of 2-5 mg. per lb. of body wt. of aureomycin or 1 mg. per lb. for terramycin, and was given once a day for 1 to 4 days. Ages of animals ranged from 3 to 14 mo. Apparent complete cure is reported in 17 of 21 cases treated with aureomycin and 12 of 14 cases treated with terramycin. R. P. Niedermeier

581. Disease conditions in the fallopian tubes and ovarian bursae of cattle. R. MOBERG, Royal Vet. Coll., Stockholm. Vet. Record, **66**, 6: 87. 1954.

The author studied the sexual organs of 1622 slaughtered cows of the Swedish Red and White and Swedish Highland breeds, and clinically studied 516 sterile Red and White cattle. The clinical diagnosis was verified as far as possible at slaughter. Results show changes in the oviduct and/or bursa of 251 of the slaughterhouse cattle and in 25 of the clinical cases. Sixty-nine of the 251 changes found post-mortem were due to malformation, cysts, etc., and 192 were due to disease. In the clinical cases one change was attributed to malformation and 24 to disease. R. P. Niedermeier

582. A fatal hypocalcaemia-like syndrome in dairy cows following the excess consumption of fodder-beet. A. N. WORDEN, J. BUNYAN, and J. PICKUP, Barnet. Vet. Record, **66**, 9: 133. 1954.

The author reports fodder-beet feeding for dairy cows is common in Denmark and Great Britain, but too high a feeding level of high dry matter content beets (i.e. 22-24%) caused digestive upsets and hypocalcaemia. This report concerns the sudden replacement of mangolds with fodder beets for a herd of 50 Guernsey cows. Within 24 hr. the entire herd was "down" with milk fever symptoms. The herd was treated for hypocalcaemia with calcium borogluconate, and even then 6 cows died. All cows dropped in milk production for several days. Analysis of urine of surviving animals was normal and urine was free of oxalate. Blood samples of two cows taken during recovery and after treatment contained 7.6 and 8.9 mg. Ca and 1.2 and 1.6 mg. of Mg per 100 ml. Toxicological examinations of the fodder-beets were negative. R. P. Niedermeier

BOOK REVIEWS

583. Principles of Crop Husbandry in India. RAJASEVASAKTA A. K. YEGAN NARAYAN AIYER, 2nd ed., XXVIII, 550 pages. RS. 17/8/— (Approximately $3.50). The Bangalore Printing and Publishing Co. Ltd., Bangalore City, India, 1953.

This discussion of the elementary principles of crop husbandry in India is directed to two classes of readers; first, to college students in agriculture, and second, the public. The need for such a book was badly felt by the agricultural students of India in the past.

This is the second edition of the publication. The two most important additions are: one, a note on "electro-culture" in the appendix, and the second on "hydroponics," especially in rela-

tion to manuring. Besides these there are a number of minor changes.

The book has been divided into 25 chapters, each dealing with a separate important topic such as manures, seeds and sowing, rotation, mixed cropping, weeds and weed control, dry farming, control of diseases and pests, storage, and systems of farming.

The first chapter deals with the climate. Less than 20% of the agricultural land is irrigated and the remaining depends upon the monsoons. It also deals with the ancient Hindu system of weather forecasts called the Brihatsamhita of Varahamihira.

There is a good discussion on the type of soils and how they have been formed. The Indo-Cangetic alluvium of the north is very fertile land, built by the thousands of years work of the two rivers, the Ganges and the Jumna. The various methods of irrigation used are discussed. The lack of proper drainage is a problem that results in heavy soil erosion every year.

There is a good description of the older methods of cultivation, including implements used, the irrigation procedures, manures, crop improvements, and other practices of the farm. These are compared with the new western systems. Though many people have now started using these improved methods on the farms, tractors, combines, and other types of powered machinery are not used due to the cheapness of hand labor. H. N. Mehrotre

BUTTER

584. Wisconsin's butter grading program. H. J. Weavers, Dairy and Food Div., Wis. Dept. of Agr., Madison. The Milk Prod. J., **45**, 5: 57. 1954.

The Wisconsin Legislature has passed a bill (Oct. 1953) making grade labeling of butter sold at retail in the state mandatory after July 1, 1954.

The basis for determining Wisconsin grades of butter will closely follow the Federal Standards for flavor, defects in body, color, and salt, and the relation of grade to flavor classification and total defects in body, color and salt. This should permit reciprocity between the two grading systems. J. J. Janzen

585. Errors of the second kind in organoleptic difference testing. G. A. Baker, M. A. Amerine, and E. B. Roessler, Univ. of Cal. Food Research, **19**, 2: 206. 1954.

An error of the second kind is made when differences in paired samples are not detected. In a study involving dilute sucrose solutions, it was found that the probability of making a correct choice increases linearly with the logarithm of sucrose concentration but that the relationships varied greatly with individuals of similar training. The individual variations were caused by varying threshold sensitivity. It is concluded that in detecting organoleptic differences only the best tasters should be used. A combination of tasters gives results poorer than the best but better than the worst. F. J. Doan

586. Churn. G. L. Myers. U. S. Patent 2,671,649. 1 claim. Mar. 9, 1954. Official Gaz. U. S. Pat. Office, **680**, 2: 464. 1954.

This is a small churn designed to operate on the vertical shaft of a home washing machine of the type which has a reciprocating motion. The shaft extends upward through the dasher, which operates in a closed vessel containing cream. The churn is easily inserted into and removed from the washing machine. R. Whitaker

587. The manufacture of aromatic butter. N Evenhuis. Netherlands Milk & Dairy J., **7**: 240. 1953.

Adequate diacetyl formation took place when a starter was shaken with approximately 1/10 of the amount of air used in an ordinary churn. The amount of O_2 could be reduced to about 1/5 of that normally present. The possible effect of a partial vacuum on the formation of oxidized substances during churning was not studied. W. C. van der Zant

CHEESE

588. Studies of the manufacture of cream-type cheese made from condensed cream. P. E. Johnson and H. C. Olson, Okla. Agr. Expt. Sta., Stillwater. Tech Bull. T-47. 1953.

A satisfactory cream-type cheese can be made by condensing low fat cream (15% fat), ripening with culture, adding stabilizer and salt, pasteurizing and homogenizing. For best results the finished product should contain about 34% fat. 17% M.S.N.F., 1% salt, 0.5% stabilizer and 52.5% T.S. The titratable acidity should be about 1%. R. W. Hunt

589. Cost of manufacturing cheddar cheese in New York State. F. V. Kosikowsky, Dept. of Dairy Ind., Cornell Univ., Ithaca, N. Y. The Milk Prod. J., **45**, 5: 38. 1954.

A study of expense statements from 4 companies representing 15 cheese factories in the New York area pool indicates that the average cost of making cheddar cheese in New York State factories during 1953 has increased almost 2.5 times since 1942.

Cheese yields obtained during the same period of time compared to yields obtained 11 years ago have remained unchanged when figured on the basis of 3.5% milk, and are significantly lower than calculated yields. J. J. Janzen

CONDENSED AND DRIED MILKS; BY-PRODUCTS

590. Modified proteins for stabilizing latex paints. K. S. Ronai and S. M. Weisberg, Natl. Dairy Research Lab., Inc., Oakdale, L. I., N. Y. Ind. and Eng. Chem., **46**, 4: 774. 1954.

The stabilizer in a latex paint, although quantitatively a minor constituent, plays an important role in controlling viscosity and freeze-thaw stability, brushing and leveling, and good pigment dispersion. Protein stabilizers sometimes react with polyvalent cations in the paint to cause objectionable viscosity increases. Controlled fragmentation of casein by enzymatic hydrolysis increased calcium compatibility thus producing good viscosity stability. The treatment also increased the protective colloidal action of the casein stabilizer and produced improved freeze-thaw stability in the finished paint. Suitable modification of casein was demonstrated by treatment with ammonia or a reducing agent as well as with an enzyme. When properly digested casein stabilizers were used, latex paints of stable viscosity could be formulated with hard water or calcium-extended pigments and these did not require heated cars for shipment or storage. B. H. Webb

591. Greater nonfat dry milk sales. ANON. The Milk Prod. J., **45**, 5: 52. 1954.

Some of the advantages of using the new bread-making process known as the 'ADMI Stable Ferment Process' are presented. The ferment is essentially a homogenous mixture containing water, yeast, yeast food, malt, sugar, and nonfat dry milk solids.

Primary gain in the use of this process is the elimination of the sponge operation in bread baking, which will save the baker money, time, and space in his operation besides a 'hoped-for' increase in the use of nonfat milk solids. J. J. Janzen

592. Wei-eiwit in brood, een theoretische beschouwing (Whey proteins in bread, a theoretical discussion). B. S. J. WÖSTMANN. Conserva, **1**: 315. 1953.

The advantages of adding whey protein to bread both from economic and nutritional point of view are discussed. W. C. van der Zaal

593. Quick and accurate testing of whey solids. ANON. The Milk Prod. J., **45**, 5: 40. 1954.

The use of a small refractometer is a quick method for testing whey solids used in cheese food manufacture.

The average accuracy of the instrument was minus 1.38% with extremes in readings of from plus 1.32% to minus 5.73%. Under certain conditions a correction factor has been applied for high solids. J. J. Janzen

594. Container for dispensing liquids. M. K. HAHN. U. S. Patent 2,675,945. 2 claims. Apr. 20, 1954. Official Gaz. U. S. Pat. Office, **681**, 3: 750. 1954.

A container for holding a can of condensed or evaporated milk is described, consisting of two pieces. The bottom is cylindrical in shape and holds the can; the top is threaded to the bottom and, when screwed on, a sharpened outlet tube pierces the can and serves as a pouring spout. R. Whitaker

DAIRY BACTERIOLOGY

595. Nebacetin—dets udskillelse og virkning i maelken (Nebacetin—its secretion and effect in milk). A. JUL OVERBY. Royal Vet. and Agr. Coll., Copenhagen. Maelkeritidende, **6**: 125. 1954. (English summary).

The use of nebacetin, an antibiotic consisting of a mixture of neomycin and bacitracin, as a preventive treatment for mastitis in injured teats, raised the question of its effect on acid production by lactic acid starters in milk. From 0.05-20 micrograms of nebacetin were added to milk together with 1% of a commercial starter. After 9 hr. at 22° C., the sample containing 0.2 micrograms showed begining inhibition of acid production. Incubation for 24 hr. at 22° C. showed that 1.0 micrograms was necessary before any inhibition became apparent. Twenty micrograms caused complete inhibition after both 9 and 24 hr. incubation. Addition of from 5-10% of milk from treated cows was sufficient to cause some inhibition. The amount of nebacetin containing milk depended on the method of treatment and the concentration of nebacetin used.

T. Kristoffersen

596. Ropy Milk. I. The biology of rope production. D. L. JONES, Dept. of Biol. Sci., Duquesne Univ., Pittsburgh, Pa. Food Research, **19**, 2: 246. 1954.

Three of 440 cultures isolated from 3500 samples of raw milk, exhibited stable ropy characteristics. These proved to be *Alcaligenes viscous* (Adametz), *Aerobacter aerogenes* strains, and *Staphylococcus cremoris-viscosus*.

F. J. Doan

597. Ropy Milk. II. The influence of physicochemical environment on the formation of ropy substance. D. L. JONES, Dept. of Biol. Sci., Duquesne Univ., Pittsburgh, Pa. Food Research, **19**, 2: 250. 1954.

Using three stable ropy milk cultures, maximum ropiness was obtained at 25° C. Surface tension, osmotic pressure, reaction, and oxygen variations within reasonable ranges did not significantly affect the ropy characteristic of the cultures. *A. viscous* and *S. cremoris* produce rope by exoenzymatic attack on milk albumen. In contrast, any medium supporting growth of *A. aerogenes* will become ropy.

F. J. Doan

598. Ropy Milk. III. Chemical studies of ropy substance. D. L. JONES, Dept of Biol. Sci., Duquesne Univ., Pittsburgh, Pa. Food Research, **19**, 2: 254. 1954.

Ropiness produced by *A. viscous* and *S. cremoris* results from degradation of milk albumin,

whereas in *A. aerogenes* cultures it results from a synthesis of a nucleoprotein. F. J. Doan

599. The role of saffron and nutmeg in indigenous milk preparations. J. V. Bhat, R. Broker, and V. Iyer, Indian J. Dairy Sci., **6**, 3: 121. 1953.

Aqueous extracts of both saffron and nutmeg were effective in controlling mesophilic spore-forming bacilli in milk. An increased generation time was observed. Both substances revealed a stimulating effect on gastric digestion.
W. C. van der Zant

600. The detection of preservatives in milk by a direct lactose fermentation test with special reference to bromoacetic acid derivatives. D. A. A. Mossel and J. G. Mandersloot. Netherlands Milk & Dairy J., **7**: 219. 1953.

Although the use of any preservative in milk is prohibited by the Netherlands Food Law, a few cases have been reported where bromacetic acid derivatives have been used to improve the keeping quality of milk. A microbiological method is presented for the detection of these preservatives. The suspected sample is inoculated with *Aerobacter aerogenes* (10^6 cells per ml. milk), transferred to Einhorn tubes and incubated at 37° C. for 20-24 hr. The levels of detection of preservatives (50% reduction of gas volume) varied from 0.2% for chloramine to 0.0001% for ethyl monobromoacetate.
W. C. van der Zant

601. The influence of time and temperature of plate incubation upon bacterial counts of market milk and related products, particularly after holding under refrigeration. F. E. Nelson and M. P. Baker, Iowa Agr. Expt. Sta., Ames. J. Milk and Food Technol., **17**, 3: 95. 1954.

A general decrease in bacterial counts on milk samples was noted when the incubation periods varied from 3 to 10 d.; 3 d. at 25° C., 4 at 21° C., 7 at 10°, and 10 d. at 5° C. The counts were higher when incubated at 25° C. for 3 d., while many bacteria did not develop colonies at 35° C.

A low coliform count is no assurance of the freedom of psychrophilic bacteria. Incubating the plates at 21 or 25° C. for 3 d. is recommended for milk with a high bacterial count due to growth during refrigeration.
H. H. Weiser

602. Bacteriological studies of a farm bulk milk handling system. E. H. Marth, J. E. Hunter, and W. C. Frazier, Dept. of Bact., Univ. of Wis., Madison. J. Milk and Food Technol., **17**, 3: 86. 1954.

Using the swab technique to determine the general sanitation on the cooling tanks, the results show that the flat open surfaces of the tank assemblies were maintained satisfactorily. However, the valve surfaces required special attention in cleansing and sanitizing.

The agitation of the milk in the cooling tanks varied from 60 to 90 sec. The time interval permitted representative sampling from the bulk tanks.

The bacterial counts on the milk held in the bulk tanks for 2 d. were comparable to the milk held 1 d. There was no apparent change in the quality of the milk cooled at a slower rate after 2 d. storage. The bacterial counts of the milk did not show any appreciable increase during the tanker transport. H. H. Weiser

DAIRY CHEMISTRY

603. Quantitative determination of amino acids using monodimensional paper chromatography. J. F. Roland, Jr., and A. M. Gross, Biochem. Research Dept., The Armour Laboratories, Chicago, Ill. Analyt. Chem., **26**, 3: 502. 1954.

A simple and convenient method for quantitative amino acid analysis of milligram or smaller portions of peptides and proteins was developed and routinely used with success. Two monodimensional solvent systems, 2-butanol-3% ammonia (3 to 1) and 72% phenol resolved 16 amino acids sufficiently well for quantitative determination by direct photometry of ninhydrin spots. Histidine and tryptophane were resolved by other systems. Analytical data on the amino acid composition of crystalline proteins such as β-lactoglobulin and bovine serum albumin when determined by this method were in good agreement with previously published values. B. H. Webb

604. Preparation of dephosphorized casein by an enzymatic method. T. A. Sundararajan and P. S. Sarma. Nature, **173**, 4406: 685. 1954.

Casein was dephosphorized by phosphoprotein phosphatase obtained from ox spleen as follows: 25 g. casein and 25 g. enzyme (283.5 phosphatase units per mg. protein nitrogen) in one liter of water containing 0.001M thioglycollic acid as activator and at a pH of 6.0 adjusted by veronal acetate buffer were incubated at 37° C. After 24 hr. an additional 10 ml. enzyme was added. After about 96 hr. incubation, the pptd. protein was centrifuged, washed with H_2O, and finally with acetone and air dried. The dephosphorized casein recovered weighed 16.5 g. Various properties of the protein were determined. It is a white amorphous powder, insol. in H_2O, sol. in dil. alk., pptd. from alk. sol. at pH 6.0, contains 15.7% N and .03% P, resembles casein in arginine, histidine, cystine, and tryptophan content, and is readily attacked by proteolytic enzymes.
R. Whitaker

605. Biuret method for soluble whey proteins in nonfat dry milk solids. H. SALWIN, QM Food and Container Inst. for the Armed Forces, Chicago, Ill. Food Research, **19**, 2: 235. 1954.

A method, employing the biuret reaction, for estimating the soluble whey proteins in dry skimmilk as an index of the degree of heat treatment is described in detail, as are also precautions against interfering nonprotein substances. A correction procedure for the interfering reaction of lactose and copper is presented. F. J. Doan

606. An investigation into the riboflavin, nicotinic acid, pantothenic acid and biotin contents of Dutch market milks, determined by the microbiological method. H. HOEFLAKE. Netherlands Milk & Dairy J., **7**: 227. 1953.

Summer milk contained more riboflavin than winter milk. The important change in riboflavin content occurred during the change from stall feeding to pasture. The nicotinic acid content decreased during winter and spring, increased during the early pasture period, and reached its maximum in June. The pantothenic acid and biotin contents showed great fluctuations which are not related to the season. The lowest values for biotin and pantothenic acid were observed in April and May, and in August and September, respectively. The values for biotin ranged from 13-32 mg. per l., for pantothenic acid from 2.1-3.6 μg. per ml., and for nicotinic acid from 70-101 μg. per 100 ml. of milk. Cultured buttermilk contained less nicotinic acid and biotin than milk.

W. C. van der Zant

607. Further studies on the vitamin B_{12} activity of milk. V. SREENIVASAMURTHY, K. N. NAMBUDRIPAD, and K. V. IYA. Indian J. Dairy Sci., **6**, 3: 105. 1953.

The vitamin B_{12} and B_{12a} contents of cow, buffalo, and human milks were determined microbiologically. The major part of the vitamin B_{12} activity of cow and buffalo milk was due to vitamin B_{12a}. In human milk only about 50% of the total vitamin B_{12} activity was represented by vitamin B_{12a}. Crossbred cows showed the highest vitamin B_{12} potency and also high values for vitamin B_{12a}. The total vitamin B_{12} activity ranged from 2.7-9.0, 2.8-4.0, and 0.055-0.16 mμg. per ml. for cow, buffalo, and human milk, respectively. With different breeds, the vitamin B_{12} activity ranged from 3.9-7.2, 2.7-5.4, 3.2-4.4, 4.3-9.0 mμg. per ml. for Gir, Tharparkar, Sindhi and cross-bred cows, respectively.

W. C. van der Zant

608. Studies in physico-chemical properties of milk. IV. Adsorption of benzene vapors. B. R. PURI, M. L. LAKHANPAL, and R. P. KATYAL. Indian J. Dairy Sci., **6**, 3: 139. 1953.

Adsorption of benzene vapors by milk was almost entirely due to the fat. The degree of adsorption varied with temperature, fat globule size, and amount of fat present. A comparison of buffalo, cow, and goat milks showed that the adsorption per g. fat was greatest for goat fat and smallest for buffalo fat. It was possible to estimate the fat content of milk by determining the adsorption of benzene vapors.

W. C. van der Zant

609. Determination of total solids in milk by Richmond's formula. K. S. RAMACHANDRAN, R. VENKATESWARA RAO, and K. P. BASU. Indian J. Dairy Sci., **6**, 3: 111. 1953.

The total solids content of 1529 samples of both cow and buffalo milk was determined with the Richmond formula (TS = 0.25 G + 1.2 F) and by direct evaporation. The calculated values differed by not more than ± 1 from the gravimetric values. A special formula for buffalo milk ($TS = 0.27 \frac{G}{D} + 1.191\ F$) gave less saisfactory results. W. C. van der Zant

610. Standardization of methods for the analysis of milk and dairy products in the Netherlands. VI. The determination of the titratable acidity of milk. The scientific committee for the standardization of methods for the analysis of milk and dairy products. Netherlands Milk & Dairy J., **7**: 246. 1953.

Methods and apparatus are described for the official determination of the titratable acidity of whole milk, skimmilk, buttermilk, acidified milk, and whey. To 10 ml. of sample 0.5 ml. of a 2% ethanolic solution of phenolphthalein is added. Titration is carried out by adding from a burette a 0.1 N solution of NaOH until a light pink color appears. Differences of 0.01 ml. shall be estimated. The endpoint of the titration is determined by comparing the color to a standard prepared by adding 0.5 ml. of 0.0005% solution of basic fuchsin to a second 10 ml. sample. One standard degree (1° N) corresponds to 1 ml. 0.1 N NaOH.

W. C. van der Zant

611. The composition of Friesian milk. L. C. JANSE. Netherlands Milk & Dairy J., **7**: 199. 1953.

Regression lines for fat and solids not fat, fat and total protein, fat and lactose, and fat and casein were calculated from the analysis of 3258 samples. An increase in fat from 2.7 to 5.7% showed (a) an increase in the total rotein content of 1.1%, (b) an increase in the solids not fat content of 0.9%, and (c) a decrease in the lactose content of 0.3%.

W. C. van der Zant

612. Procedure for the separation, detection, and identification of the common vegetable gums in dairy products, with special reference to alginates. H. N. BUNDESEN and M. J. MAR-

TINEK, Board of Health, Chicago, Ill. J. Milk and Food Technol., **17**, 3: 79. 1954.

The authors have suggested a procedure for the separation and identification of vegetable gums in dairy products. The alginates are precipitated as algenic acid and then redissolved from the protein precipitate with magnesium sulfate. Alginates are identified by their color reaction with ferric sulfuric acid reagent. The gums can be identified in concentrations as low as 0.1 g. per 100 g. of product.

H. H. Weiser

DAIRY ENGINEERING

613. Device for mixing and homogenizing liquids. A. G. BORCK (assignor to Aktiebolaget Separator). U. S. Patent 2,673,075. 7 claims. Mar. 23, 1954. Official Gaz. U. S. Pat. Office, **680**, 4: 1031. 1954.

A centrifugal device is described for intimately mixing two liquids. R. Whitaker

614. Refrigeration control means. J. CALLING (assignor to International Harvester Co.). U. S. Patent 2,674,101. 5 claims. Apr. 6, 1954. Official Gaz. U. S. Pat. Office, **681**, 1: 83. 1954.

A cabinet for cooling milk in cans, having a motor driven compressor on top and submerged cooling coils. Automatic operation is obtained through the use of two circuit breakers, one on the evaporator and one on the pump circulating air over the evaporator.

R. Whitaker

615. Desiccating apparatus. D. D. PEEBLES (assignor to Western Condensing Co.). U. S. Patent 2,675,070. 2 claims. Apr. 13, 1954. Official Gaz. U. S. Pat. Office, **681**, 2: 432. 1954.

A cone-shaped spray drier is described, consisting of a centrifugal spray atomizer, which ejects the atomized particles outward into a zone of hot gas, where they are flash dried. Any semidried material adheres temporarily to the outer surfaces of a series of revolving drums, mounted vertically opposite the spray wheel. When dry, the particles drop off the drum drying surface and are discharged with the air stream from the bottom of the cone.

R. Whitaker

616. Plate type heat exchanger. N. R. G. EKWALL. U. S. Patent 2,676,000. 8 claims. Apr. 20, 1954. Official Gaz. U. S. Pat. Office, **681**, 3: 767. 1954.

Cooling in a plate type heat exchanger is facilitated by a system of parallel grooves or depressions pressed into the plates, which causes the product to flow in a tortuous pathway from inlet to outlet. R. Whitaker

617. Apparatus and method for filling milk containers. H. B. BABSON and C. A. THOMAS (assignors to Babson Bros. Co.). U. S. Patent 2,676,743. 11 claims. Apr. 27, 1954. Official Gaz. U. S. Pat. Office, **681**, 4: 1051. 1954.

A device for filling bottles with milk. The milk is drawn into a group of bottles by means of vacuum applied to the last of the series of bottles. R. Whitaker

DAIRY PLANT MANAGEMENT AND ECONOMICS

618. Paper vs. glass. C. W. HALL, Agr. Eng. Dept., Mich. State Coll., East Lansing. Food Eng., **26**, 3: 101. 1954.

In order to determine if the frequent extra charge of 1¢ per qt. for milk in paper cartons is justified, an analysis was made of filling operations in 8 dairy plants located in central and southern Mich. Taken into consideration were costs of bottles, cases, caps, hoods, and trips per bottle. Also included were labor and utilities for bottling, bottle and case washing, and clean-up operations. Cost of equipment involved was also considered. The cost of the glas operation was $0.34/100 lb. milk for 100 bpm capacity, 80,000 lb. milk/day. The cost of paper carton filling and forming operations was about $0.90/100 lb. milk for 65 cartons/min, 80,000 lb/day. This was about 1¼¢/qt more than the entire glass operation. The principal cause of the difference is the single trip of the paper carton. T. J. Claydon

619. Cost of obtaining pasteurized milk. H. B. METZGER, Maine Agr. Expt. Sta., Orono. Bull. 515. 1953.

Information concerning the methods and costs of operation of subdealer-distributors was obtained by personal interviews. A total of 27 subdealers-distributors, 25 small processor-distributors, and 17 processing plants supplying subdealers were included in the study. The results indicate that the subdealer method of processing was more expensive than in the distributor's plant. Comparison of unit costs of having milk pasteurized (including bottling, placing in refrigerator, and container cost) was 4.9¢ for a subdealer-distributor, as compared to 3.8¢ per qt. for a small processor dealer. Volume of business was important in determining costs for the subdealer-distributor. Those processing less than 500 qt. per day had a cost averaging 4.4¢, as compared to 3.4¢ for those over 500. Prices charged subdealers depended upon supplies, services rendered, size, and location of supplying dealer. Range in prices was from 16-18½¢ per qt. for standard pasteurized milk, where the milk was supplied by the dealer, and from 2-3¢ where it was not.

R. W. Hunt

620. Supply and utilization of fluid grade milk in selected Mississippi markets, 1950 and 1951. L. P. JENKINS and W. W. MOFFETT,

JR., Miss. Agr. Expt. Sta., State College. Tech Bull. 30. 1953.

The general trend of supply vs. demand worked in reverse. That is, when milk supplies were high, consumption was low. The same held true for butterfat. A surplus of fat developed during the fall and winter, while a shortage of nonfat solids developed during this same time. Fluid milk plants in the 9 markets studied purchased 135,598,000 lb. of milk in 1950 at a weighted price of $4.89 cwt. of 4% f.c.m. In 1951, the same plants purchased 143,762,000 lb. of milk at $5.48 cwt. Class II milk was purchased at the level of 14% of the total purchased in 1950, and 13% in 1951. Per capita consumption for 5 of the markets studied was found to be approx. 0.6 pt/day. The U. S. average is 0.9. R. W. Hunt

621. The market for fluid skimmilk. G. K. BRINEGAR and S. JOHNSON, Storrs Agr. Expt. Sta., Storrs, Conn. Bull. 303. 1953.

For 16 Conn. dealers, sales of fluid skimmilk have increased 535% between Jan. 1948 and June 1951. The increase was 531% between Jan. 1947 and Feb. 1953 for 45 large U. S. cities. Consumers apparently prefer a skimmilk of about 0.75% fat and 10% S.N.F. Findings indicated that skimmilk is overpriced as compared to whole milk. However, family incomes do not seem to govern consumption. Purchases of skimmilk are about the same for all income groups. R. W. Hunt

622. Bottle carrier. T. V. LUGT, JR. (assignor to Sutherland Paper Co.). U. S. Patent 2,675,-264. 10 claims. Apr. 13, 1954. Official Gaz. U. S. Pat. Office, **681**, 2: 489. 1954.

A collapsible carrier for a Pure-Pak style paper milk bottle. R. Whitaker

623. Costs, quality, and prices of fluid milk in rural and urban areas of Utah and Montana. W. M. ALLRED and E. H. WARD, Utah Agr. Expt. Sta., Logan.

Cost of processing and delivering fluid milk and other products handled by small and medium sized dairy plants located in outlying communities and urban areas was obtained from 33 milk plants in Utah and 9 in Mont. The findings were as follows: (1) Average operating costs (processing, delivery, administration) were 5.06¢/qt of milk equivalent. (A milk equivalent is based on a value of 1.00/ qt of milk; other products being assigned a value based on their processing cost as compared to this value of one). (2) Cost of processing was 2.37¢/qt of milk equivalent, accounting for 47% of all marketing costs. Delivery costs were 2.12¢ (42%), while administrative costs were 0.57¢ (11%). (3) Labor was the largest cost item accounting for 59% of operating costs, truck costs were next (13%), followed by depreciation on buildings and equipment (4%). (4) There was considerable variation in processing efficiency among plants in Utah. Six of the smallest plants had costs between 2.4 and 5¢, and three had costs above 5¢/qt. Among the 9 largest plants, 7 had less than the average cost of 2.4¢, and 2 above. Five of the middle-sized plants had costs less than 2.4¢, 7 had costs between 2.4 and 5¢, and 1 plant's costs were above 5¢. (5) On the average, the 9 largest plants processed milk for 2¢/qt less than the 11 smallest ones. However, delivery costs were nearly the same for most all plants.

R. W. Hunt

624. Pitcher. H. M. GREENWOOD (assignor to Hal Greenwood, Inc.). U. S. Patent 2,676,732. 1 claim. Apr. 27, 1954. Official Gaz. U. S. Pat. Office, **681**, 4: 1047. 1954.

A container for holding and dispensing liquids, such as evaporated milk, from a tin can, the product being poured through a sharpened beveled tube which punctures the can when the pitcher cover is applied. R. Whitaker

625. DISI's world congress for milk utilization. ANON. Sou. Dairy Prod. J., **55**, 1: 52. 1954.

More than 200 persons from 30 countries met in Washington, D. C., Nov. 20-21, 1953 to explore possible solutions of the international milk surplus-deficit problem. One very significant obstacle to increasing consumption of milk in some countries was said to be prejudices against milk. Pricing policies were recommended as a means of giving producers a fair return but not to price milk out of the market. Loans of the International Bank for Reconstruction and Development have resulted in increases both in production and in consumption of milk. It was recommended that low-income countries purchase the essentials for producing their own milk rather than importing it. Inducing the people in rich countries to consume more milk was considered as a partial solution to the surplus problem. The importance of knowledge of the principles of milk production and milk quality in under-developed areas was emphasized. Ice cream at reasonable prices was mentioned as a valuable sales product. The removal of trade barriers was suggested. The addition of powdered skimmilk to the diet of Japanese school children after World War II was cited as a good example of improvement of the diets of undernourished peoples.

F. W. Bennett

GENETICS AND BREEDING

626. Semen diluents in the artificial insemination of cattle. R. C. CAMPBELL and J. EDWARDS. Nature, **173**, 4405: 637. 1954.

A comparative study is described wherein the relative merits, as measured by the conception rate, were determined of the two bull semen diluents (1) egg yolk citrate and (2) egg yolk phosphate buffer when used with combina-

tions of 3 bacteriostatic agents, (3) sulphanilamide, (4) penicillin, and (5) streptomycin. (1) + (4) was 9.3% better than (1); (1) + (3), (1) + (4), and (1) + (5) surpassed (1) by 6.8%; and (2) was 5.5% better than (1). The authors claim the only combinations of real economic importance are (1) + (3), (1) + (4), and (1) + (5). R. Whitaker

627. Osmotic pressure of bull semen diluents. R. H. Barnes. Nature, **173**, 4405: 636. 1954.

Data are given on the freezing point depression (Δ) of bull seminal plasma and semen diluted with egg yolk and phosphate buffer, and egg yolk and citrate buffer, with various combinations of bacteriostatic and/or bactericidal substances. The Δ of the plasma is $-.53$ and it seems desirable to maintain the same osmotic pressure when diluents are employed. The composition of egg yolk mixtures isotonic with bull seminal plasma is given. The use of penicillin and streptomycin instead of sulphanilamide has practically no effect on Δ.

R. Whitaker

HERD MANAGEMENT

628. Timing milk production for highest profit in the Detroit milkshed. E. E. Peterson, Mich. Agr. Expt. Sta., East Lansing. Special Bull. 386. 1953.

As a result of use of the base-excess price plan in the Detroit area, it was found that net farm incomes could be raised $500 and $1,000 for better-than-average operators whose herds contained 10 and 20 cows, respectively. For farms whose production decreased as a result of attempts to even out annual production, the period of highest milk production coinciding with that of best pasture conditions was likely to be most profitable.

The principal problems which must be solved in reducing seasonality of milk production are: (a) natural tendency toward spring milk production; (b) seasonal variation in the kind and quality of feed; (c) weather; (d) insects; and (e) uncertainty as to the outcome of the change.

Important methods for reducing seasonality of milk production are: (a) planned breeding program; (b) use of improved harvesting methods to improve forage quality; (c) heavier feeding of concentrates in the late summer and early fall; (d) adequate housing; (e) access to shade and water in hot weather; and (f) control of insects. The amount of knowledge and managerial ability required to achieve even production was nearly the same regardless of size of herd. R. W. Hunt

629. Lactation studies. V. Causes in variations in fat percentage. R. E. Erb, M. M. Goodwin, W. N. McCaw, R. A. Morrison, and A. O. Shaw, Wash. Agr. Expt. Sta., Pullman. Sta. Cir. 229. 1953.

Records on 19 Holstein cows milked two and three times daily were available. Every milking was weighed and tested for a 305-d. lactation period. In addition, 61 cows were followed for a 10-d. period. To study outside differences 2,491 D.H.I.A. records were summarized. The results indicate that (1) fat percentage of milk commonly varies as much as 2% from one milking to the next on an individual cow; (2) day-to-day variation in average fat percentage of a group of 9 or 10 animals will frequently exceed 0.5%. When as many as 61 cows were involved, the variation did not exceed 0.2% over a 10-d. period; (3) fat percentage increases rapidly during the last few mo. of a lactation, especially if the calf is carried more than 200 d.; (4) Holsteins have a distinctly different fat percentage lactation curve than Jerseys or Guernseys.

R. W. Hunt

630. Lactation studies. VI. Improving the accuracy of longer testing intervals and the accuracy of current methods. R. E. Erb, M. M. Goodwin, W. N. McCaw, R. A. Morrison, and A. O. Shaw, Wash. Agr. Expt. Sta., Pullman. Sta. Cir. 230. 1953.

Several suggestions are made for improving the accuracy of records kept on lactating cows.

R. W. Hunt

631. Barn gutter cleaner. A. F. Klinzing. U. S. Patent 2,672,970. 10 claims. Mar. 23, 1954. Official Gaz. U. S. Pat. Office, **680**, 4: 1000. 1954.

Refuse is cleaned from barn gutters by a screw conveyor type of cleaner.

R. Whitaker

632. Calf weaning halter. E. D. Maynard. U. S. Patent 2,672,846. 4 claims. Mar. 23, 1954. Official Gaz. U. S. Pat. Office, **680**, 4: 965. 1954.

A description is given of a halter for the head of calves with several prongs positioned on the nose of the animal. R. Whitaker

633. Reciprocating hoe type manure conveyor. V. L. Miller. U. S. Patent 2,675,121. 5 claims. Apr. 13, 1954. Official Gaz. U. S. Pat. Office, **681**, 2: 447. 1954.

A device for moving litter in the gutter of barns to the end and discharging it outside through an opening in the side of the barn.

R. Whitaker

634. Reversible calf table. G. D. Bentley. U. S. Patent 2,674,980. 2 claims. Apr. 13, 1954. Official Gaz. U. S. Pat. Office, **681**, 2: 406. 1954.

A device for holding farm animals rigidly, which may be tilted for convenience in working on an animal. R. Whitaker

635. Milk release apparatus. T. W. Merritt and C. A. Thomas (assignors to Babson Bros.

Co.). U. S. Patent 2,674,979. 7 claims. Apr. 13, 1954. Official Gaz. U. S. Pat. Office, **681**, 2: 405. 1954.

A complete milking system is described, consisting of a vacuum milker, operating on a cow in a milking stall, an arrangement of pipe lines and equipment for continuously releasing the milk from reduced pressure to atmospheric, and delivering the milk into a tank at a lower level than the cow. R. Whitaker

636. Southeastern S. Dak. farm record summary. ANON., S. Dak. Agr. Expt. Sta., College Station. Agr. Econ. Pamphlet No. 48. 1953.

This is the tenth annual report of the farm record study in S. E. S. Dak. R. W. Hunt

637. North Central S. Dak. farm record summary. ANON., S. Dak. Agr. Expt. Sta., College Station. Agr. Econ. Pamphlet No. 45. 1953.

This is the tenth annual report of the farm record study in N. C. S. Dak.
R. W. Hunt

638. Milking machine pulsator. L. T. CONDE. U. S. Patent 2,675,821. 1 claim. Apr. 20, 1954. Official Gaz. U. S. Pat. Office, **681**, 3: 713. 1954.

A piston activated slide valve type of pulsator for operating a vacuum milker is described. R. Whitaker

639. Milking machine. E. DOMINGO. U. S. Patent 2,677,348. 6 claims. May 4, 1954. Official Gaz. U. S. Pat. Office, **682**, 1: 129. 1954.

Details are given for a vacuum operated pulsator milking machine. R. Whitaker

640. Milker system. C. A. THOMAS (assignor to Babson Bros. Co.). U. S. Patent 2,676,560. 6 claims. Apr. 27, 1954. Official Gaz. U. S. Pat. Office, **681**, 4: 997. 1954.

A valve for placing in the vacuum line of a milking system which admits air when the vacuum reaches a predetermined level. The operation of the valve is easily visible to the machine operator from all directions.
R. Whitaker

ICE CREAM

641. Low carbohydrate ice cream for diabetics. C. G. ROBERTS and C. D. DAHLE, Pa. State Coll. Ice Cream Trade J., **50**, 4: 66. 1954.

Formulas for diabetic ice cream and a method of calculating the calorie value of diabetic ice cream are presented. W. H. Martin

642. Time and motion studies in ice cream plants. F. M. SKELTON, General Ice Cream Corp., Schenectady, N. Y. Ice Cream Trade J., **50**, 4: 26. 1954.

Time and motion studies and an analysis of data obtained on flow process charts have usually resulted in an improvement in plant operation. Production per labor hr. in mix making was increased 30%; freezing, 26%; and cup making, 102%, when the 1949 and 1952 production figures were compared. W. H. Martin

643. Combination package. R. O. TARDIFF (assignor to Breyer Ice Cream Co.). U. S. Patent Reissue 23,806. 2 claims. Mar. 23, 1954. Official Gaz. U. S. Pat. Office, **680**, 4: 895. 1954.

A package for holding two ice cream pies or tarts, as described previously in U. S. Patent 2,617,577. R. Whitaker

644. Multiflavor ice cream nozzle. R. F. ANDERSON. U. S. Patent 2,673,675. 10 claims. Mar. 30, 1954. Official Gaz. U. S. Pat. Office, **680**, 5: 1274. 1954.

A filling head for placing several flavors of ice cream in cartons. R. Whitaker

645. Apparatus for the production of ice cream sandwich wafers. D. A. E. BEER. U. S. Patent 2,673,531. 4 claims. Mar. 30, 1954. Official Gaz. U. S. Pat. Office, **680**, 5: 1231. 1954.

A device for assembling ice cream sandwiches from wafers and cut brick ice cream, the whole installed in a refrigerated chamber.
R. Whitaker

646. Coating composition for frozen confections. P. J. CARTER (assignor to Atlas Powder Co.). U. S. Patent 2,674,534. 11 claims. Apr. 6, 1954. Official Gaz. U. S. Pat. Office, **681**, 1: 211. 1954.

A coating for frozen novelties consisting of a vegetable oil phase, emulsified into a continuous aqueous phase. The oil selected is solid at ice cream storage temperatures and has a melting point of 75-115° F. An emulsifier is also employed, composed of an ester with an HLB value in the range of 9.5-11.5.
R. Whitaker

647. Machine for manufacturing frozen confections. C. K. NELSON and C. R. BEHLING (assignors to Eskimo Pie Corp.). U. S. Patent 2,677,943. 5 claims. May 11, 1954. Official Gaz. U. S. Pat. Office, **682**, 2: 372. 1954.

Frozen confections are hardened by conveying them upward on an endless chain conveyor in contact with a set of coils or pipes containing a refrigerant and then downward in contact with another refrigerating unit, the whole enclosed in an insulated box. R. Whitaker

648. Ice cream cone vending machine. H. HEINIG. U. S. Patent 2,678,250. 12 claims. May 11, 1954. Official Gaz. U. S. Pat. Office, **682**, 2: 462. 1954.

Factory filled ice cream cones are dispensed one at a time through a small door from a vertical endless conveyor equipped with cone holding pockets. R. Whitaker

649. Frozen pop confection. R. H. Sueskind. U. S. Patent 2,677,615. 6 claims. May 4, 1954. Official Gaz. U. S. Pat. Office, **682**, 1: 206. 1954.

A frozen ice or ice cream stick novelty consisting of a molded block of the product on a stick with a layer of cake impaled on the stick and held against the product. The layer of cake is larger than the block of product and, being of the absorbent type, catches any drippings as the piece is consumed. R. Whitaker

MILK AND CREAM

650. A modified rapid solvent extraction method for estimation of WIA in cream. T. V. Armstrong and W. J. Harper, Dept. of Dairy Technol., Ohio State Univ., Columbus. The Milk Prod. J., **45**, 5: 37. 1954.

A simple, rapid, solvent extraction procedure has been developed for the estimation of the WIA content of cream.

The procedure is as follows: Add 5 ml. of 95% ethyl alcohol to 10 g. of cream in a 125-ml. glass-stoppered separatory funnel. Shake vigorously for 30 sec. Add 40 ml. pet. ether and shake for 1 min. Let stand for 3 min. and drain off the alcohol-serum portion. Add 25 ml. distilled water, shake gently 5× and after 30 sec. drain off the lower layer. Empty contents of funnel into a 125-ml. Erlenmyer flask. Rinse the funnel with 10 ml. pet. ether. Titrate with 0.025N sodium ethylate, using 1 ml. of 1% phenolphthalein in absolute alcohol.

The WIA values are generally expressed in mg/100 g. fat. The following equation permits the calcuation: mg. WIA/100 g. fat $= 6.75 \times \frac{\text{ml. sodium ethylate}}{\text{wt. fat in sample}} \times 100$.

The time required for performing single samples varied from 8-10 min. J. J. Janzen

651. A study of mixed milk sample methods of sediment testing using off-the-bottom standards and equipment for possible use with farm bulk milk holding tanks. B. J. Liska and H. E. Calbert, Dept. of Dairy and Food Ind., Univ. of Wis., Madison. J. Milk and Food Technol., 17, 3: 82. 1954.

A satisfactory sediment test can be secured from farm bulk tanks by using a 1 qt. mixed milk sample and concentrating the sediment on ¼ the area of a standard sediment disc. Off-the-bottom sediment standards can be used for evaluating the grades of these modified discs. The adapted tests results are comparable to those obtained by the standard off-the-bottom method on 10-gal. cans of milk.

H. H. Weiser

652. Process for preserving the fresh natural flavor of butterfat. G. D. Turnbow (assignor to International Dairy Engineering Co.). U. S. Patent 2,673,155. 34 claims. Mar. 23, 1954. Official Gaz. U. S. Pat. Office, **680**, 4: 1051. 1954.

Cold raw milk is separated to form skimmilk, which is dried, and cream. If cream is of high fat content (80%), heat 115-125° F.; otherwise avoid heating, and add a sugar solution composed of lactose, sucrose and glucose to inhibit off flavors induced by heating fat. Pasteurize in the range of 143-190° F. If of high fat content, chill and place in storage; if of low fat content, separate after reversing phases and store dry fat and add nonfat portion to previous skimmilk for drying. R. Whitaker

653. Milk substitute (mineral-enriched meat). H. B. Lockhart (assignor to Swift & Co.). U. S. Patent 2,673,803. 2 claims. Mar. 30, 1954. Official Gaz. U. S. Pat. Office, **681**, 1: 1311. 1954.

A substitute for milk consisting of finely ground meat, such as beef, lamb, pork, etc., to supply the protein fraction, fat, such as lard, vegetable oil, etc., carbohydrate, consisting of tapioca or potato flour, sucrose, lactose, etc., and combinations of minerals, such as calcium phosphate, gluconate, carbonate, magnesium acetate or citrate, magnesium chloride, ferrous sulphate, ferric ammonium citrate, potassium chloride, potassium phosphate, sodium chloride, etc. R. Whitaker

654. Centrifugally operable valve ring for power washing cream separators. K. C. Tregillus (assignor to Vernay Laboratories, Inc.). U. S. Patent 2,674,254. 9 claims. Apr. 6, 1954. Official Gaz. U. S. Pat. Office, **681**, 1: 129. 1954.

An opening around the outside edge of a separator bowl is sealed by a flexible ring of rubber or similar material. The ring expands and contracts in relation to the centrifugal force, normally sealing the opening when the bowl is at full speed and unsealing the opening to allow for washing at subnormal speeds.

R. Whitaker

655. Onderzoekingen betreffende de methyleenblauw — proef. II. Kan het keren van de reductasebuizen vervangen worden door het toevoegen van stremsel? (Some investigations on the methylene blue reduction test. II. Is it possible to avoid the inversion of the tubes by the addition of rennet?) Th. E. Galesloot. Netherlands Milk & Dairy J., 7, 204. 1953.

The addition of too much rennet presented difficulties in reading the tubes because of whey separation. Smaller amounts of rennet, which did not cause whey separation, did not prevent the formation of a cream layer on the sample. Hourly inversion without the addition of rennet was preferred. W. C. van der Zant

656. Temperatuur metingen tydens het pasteuriseren van melk in een klokpasteur (The measurement of temperature when pasteurizing milk in a steel cap pasteurizer.) H. Mulder and S. Broersma. Netherlands Milk & Dairy J., **7**: 208. 1953.

The pasteurizer consists of a steel cap which is lowered over a carrier with bottles of milk. Steam is injected through a perforated plate which supports the carrier. This pasteurizer proved satisfactory for pasteurization at high temperatures but less suitable at lower temperatures because of considerable differences in temperature between bottles located at different places in the pasteurizer.

W. C. van der Zant

657. Investigations into the variations of quantity and quality of milk from different quarters of udder. H. Singh and C. N. Dave. Indian J. Dairy Sci., **6**, 3: 97. 1953.

Five Sindhi cows were milked daily at the same hour and order of milking by the same milker. Milking by individual teat or front and hind quarters in any order essentially had no effect on the total milk yield. Hind quarters yielded 33% more milk than front quarters. Right quarters yielded 12.2% more milk than left quarters irrespective of the order of milking. The right hind teat commonly gave higher yields. W. C. van der Zant

658. Calculations of the solids-not-fat contents of cow and buffalo milk. A. M. El-Sokkary and H. A. Hassan, Fouad Univ., Cairo. Indian J. Dairy Sci., **6**, 3: 93. 1953.

Total solids and solids-not-fat were calculated for each of 100 samples of cow and buffalo milk with Richmond's formula. These values compared favorably with those obtained by determining the total solids content by direct evaporation. W. C. van der Zant

659. Dairy product and process. A. D. Maissian. U. S. Patent 2,671,025. 4 claims. Mar. 2, 1954. Official Gaz. U. S. Pat. Office, **680**, 1: 222. 1954.

A food product consisting of whole milk solids and a cereal product. The cereal product is first roasted with butter, then aded to either milk or light cream and heated, aerated, and held hot for about 2 hr. After cooling and holding for a day, the liquid portion is drained off and the solid material refrigerated until consumed by infants or adults on a reducing diet.

R. Whitaker

MILK SECRETION

660. Rate of milk secretion in dairy cattle in the intervals between consecutive milkings. G. L. Bailey, P. A. Clough, and F. H. Dodd. Nature, **173**, 4400: 404. 1954.

Variations in the residual milk, that was left in the secretory tissues of the udder after milking, was an unappreciated factor in previous studies on the rate of milk secretion. To eliminate this effect, cows were milked at intervals of 3, 6, 9, 12, 15, and 18 hr., the order being 12(6), 9(5), 15(5), 6(8), 18(5), 3(25), 18(6), 6(10), 15(8), 9(7), and 12(6), the figures in brackets indicating the number of consecutive milkings for each interval. Using this system, it was shown that both milk and fat secretion decline with increasing interval since the last milking. The rate of decline of fat secretion is not greater and may be less than the decline in milk secretion. R. Whitaker

NUTRITIVE VALUE OF DAIRY PRODUCTS

661. The effects of antibiotics in milk on the human intestinal coliform bacteria. E. I. Stoltz and D. J. Hankinson, Dept. Dairy Ind., Univ. of Mass., Amherst. J. Milk and Food Technol., **17**, 3: 76. 1954.

The concentrations of 0.1 mg. (50 mg. per day) of streptomycin per ml. of milk and 0.25 mcg. (125 mcg. per day) of aureomycin per ml. of milk markedly reduced the number of human intestinal bacteria in 10 human subjects. Penicillin reduced the intestinal flora but not during antibiotic ingestion by two subjects.

The authors conclude that a decrease in the normal intestinal flora by antibiotics may cause a decrease in the synthesis of beneficial vitamins by the growth of bacteria in the human intestinal tract. H. H. Weiser

662. Nutritive value of milk proteins. II. Stability during sterilization of evaporated milk as determined by the rat growth method. A. Z. Hodson, Research Labs., Pet Milk Co., Greenville, Ill. Food Research, **19**, 2: 224. 1954.

By means of the rat growth method some losses in nutritive value of the proteins of evaporated milk were demonstrated. These concerned principally the sulfur-containing amino acids, cystine, cysteine, and methionine and were largely a result of sterilization. The author argues rather conclusively that these nutritive losses, while of significance for rats, are of no significance in human nutrition.

F. J. Doan

PHYSIOLOGY AND ENDOCRINOLOGY

663. The effect of an iodinated casein fraction on the milk yield of goats. J. Weits, A. C. Houtman, and J. R. Roborgh. Netherlands J. of Agr. Sci., 1: 39. 1953.

The effect of iodinated casein and the thyroxine-free lactogenic factor from hydrolyzed iodinated casein was investigated on lactating goats. Increased milk yields decreased rapidly when the injections were discontinued. During the administration a decrease in weight of 10% was observed; heart rate and body temperature

were increased. The results with fraction B were not more favorable than those obtained with iodinated casein. W. C. van der Zant

664. Progesterone and relaxin as aids in ova transfer. A. E. Dracy, S. Dak. Agr. Expt. Sta., Brookings. 66th Ann. Rpt., p. 130. 1953.

Readjusting the heat periods with progesterone appears to have no ill effects on normal fertility. This conclusion is based on recovery of fertilized ova. There is a possibility that recycling the estrus period of cows can be regulated so that a cow can be brought in heat and bred within a predicted time. This may prove advantageous for artificial insemination. R. W. Hunt

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